

# Census of Amphibians and Fishes in Lentic Habitats of Lassen Volcanic National Park: A Report to the National Park Service

LVNP Study Number: LAVO-00717



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April 15, 2005



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## Abstract

We conducted a cooperative inter-agency investigation into the status of native amphibians and nonnative fishes in lentic habitats at Lassen Volcanic National Park. Census surveys at Lassen Volcanic National Park were conducted during summer 2004, and results of surveys that we conducted in the Thousand Lakes and Caribou Wilderness areas during summer 2002 are also presented here. Visual encounter surveys were conducted to identify presence, species, life stage and relative abundance of amphibians, and timed gill net sets or visual surveys were used to identify fish presence, species, and relative abundance. Habitat data were also collected. We analyzed the data in two different ways. One analysis uses the Mann-Whitney U test and multinomial logistic regression to examine attributes of historically stocked lakes potentially contributing to the observed presence or absence of fish. In the second analysis we use generalized additive models to examine site attributes associated with amphibian occurrences, including the presence or absence of fish. Herpetofauna detected include the Pacific treefrog (*Hyla regilla*), western toad (*Bufo boreas*), long-toed salamander (*Ambystoma macrodactylum*), Cascades frog (*Rana cascadae*), rough-skinned newt (*Taricha granulosa*), western terrestrial garter snake (*Thamnophis elegans*), and the common garter snake (*T. sirtalis*). Fish species detected include three species of trout (Family: Salmonidae), five minnow species (Family: Cyprinidae), and the Tahoe sucker (*Catostomus tahoensis*). Of the confirmed historically stocked lakes at LVNP, both the number of inlets present, and size of water bodies (both perimeter and area), was significantly greater at lakes that continue to support fish, while the elevations were significantly lower, compared to those sites that have gone fishless. The model best able

to predict which lakes would revert to a fishless condition included only the number of inlets and perimeter. Our models suggest that populations of long-toed salamanders and Pacific treefrogs are less likely to be found in water bodies supporting fish. Fairy shrimp also appear to be negatively affected by fish. The Cascades frog was only detected at three sites, and could not be analyzed statistically. We believe that the Cascades frog is in immediate risk of extirpation from the Lassen region. Although fish clearly adversely affect palatable amphibian species, a number of considerations lead us to believe that fish are not the main driver behind the observed regional decline of Cascades frog. Factors outside the scope of this investigation, such as pollution and disease, may play an important role in declines observed in the Lassen region.

## Introduction

Amphibian declines have been recognized as a global phenomenon (Blaustein and Wake 1990). Particularly alarming are declines from within protected areas, where habitat has been preserved in a relatively natural state (Bradford 1991; Bradford et al. 1994; Bosch et al. 2001). The decline of Cascades frog (*Rana cascadae*) from Lassen Volcanic National Park (LVNP) was first documented in the early 1990's (Fellers and Drost 1993), although the exact mechanism of the decline remains unknown. Potential causative factors that have been implicated in the decline of amphibians include habitat destruction (Dodd and Smith 2003), disease (Blaustein et al. 1994; Berger et al. 1998; Bosch et al. 2001; Fellers et al. 2001), increased ultraviolet radiation (Blaustein et al. 2003), climate change (Pounds and Crump 1994; Pounds et al. 1999), the downwind drift of airborne chemicals from agricultural areas (Davidson 2004; Fellers et al. 2004), and the introduction of fish into historically fishless habitats (Knapp and Matthews 2000; Matthews et al. 2001; Knapp 2005; Welsh et al. in review).

The focus of this report is to document the status of native amphibians and nonnative fishes in LVNP with a goal of determining if nonnative fish have a role in the decline of some species of amphibians in LVNP. Fish stocking was gradually phased out at LVNP during the 1970's. While some of the water bodies previously stocked have since reverted to a fishless condition, others now support self-sustaining fish populations. In addition to trout, various other species of fish have become established in LVNP, released by anglers in pursuit of trout. Prior to this investigation, LVNP had not had a formal inventory of lentic fish or amphibian populations. Given the previously

documented decline of Cascades frog, the unknown status of other lentic-breeding amphibians, and the recently changed but likely stabilized distribution of fishes, it was determined that a rigorous quantitative survey of amphibian and fish populations in available lentic habitats was needed to guide preservation and management of aquatic biodiversity at LVNP.

The investigation conducted at LVNP and described in this report is the result of a collaborative effort between the US Forest Service, Pacific Southwest Research Station, Redwood Sciences Laboratory (RSL), and the Klamath Network of the National Park Service, Southern Oregon University, and LVNP. This investigation includes an invertebrate component, which is still in progress and will be reported on separately. Prior to the initiation of the investigation, RSL conducted a similar study in many wilderness areas of northern California, in collaboration with the California Department of Fish and Game. During the summer of 2002 field crews from RSL surveyed all lentic habitats within the Thousand Lakes Wilderness and a substantial portion of the Caribou Wilderness. Caribou Wilderness adjoins the eastern border of LVNP, and the Thousand Lakes Wilderness is located just over ten kilometers north of the Park. Habitats in these two wilderness areas are similar to some of the habitats found in LVNP. The intent of this report is to present the results of the 2004 survey of LVNP. However, results of surveys in the Thousand Lakes and Caribou Wilderness areas are relevant, both ecologically and from a management perspective, to LVNP. Therefore, we have included relevant results of surveys conducted in the two proximal wilderness areas in this report,

mostly in a more summary format than the results of the surveys conducted within LVNP.

Surveys at LVNP were conducted under Scientific Research and Collecting Permit Number LAVO-2004-SCI-0015, issued by LVNP.



## **Methods**

### **Field Surveys**

The methods presented in this section provide an overview of how and what data was collected in the field. For additional information see Appendix A, which contains the survey protocol used at LVNP.

#### Lassen Volcanic National Park

Surveys at LVNP, led by Jonathan Stead of RSL, were conducted between June 3 and August 18, 2004. The six-person survey crew also included Miranda Haggarty (also of RSL), and John Speece, Kate Meyer, Jesse Goldstein and Lyndia Hammer, from Southern Oregon University. All lentic habitats found on 7.5 minute USGS Topographic Quadrangles within the boundaries of LVNP were visited, plus any unmapped sites discovered incidentally. UTM coordinates were collected at all surveyed sites using hand-held GPS units in the NAD27 datum, and any unmapped locations were later added to the GIS database. A unique site identification number was assigned to each site, based upon the CalWater (California Interagency Watershed Map of 1999) database. If a site had a previously designated CalWater identification number, that number was used. Otherwise, a new number was assigned by adding a decimal to the nearest site that did have a CalWater identification number.

Timed visual encounter surveys, of shoreline and wadeable habitats, were conducted to identify presence, species, life stage and relative abundance of amphibians. Amphibians

were captured, with small aquarium nets, only when necessary to confirm species identification. In addition to amphibians, similar data was collected for reptiles (typically *Thamnophis* spp.) and fairy shrimp (Order: Anostraca) encountered during surveys. Timed gill net sets or visual surveys were used to identify fish presence and species. Gill net surveys included a measure of catch per unit effort (where effort is time), which can be used as an estimate of relative fish density. Nets (35 meter-long, mixed-mesh, nylon monofilament, sinking gill nets) were set whenever fish were visually confirmed to be present in a water body, or whenever a visual survey was insufficient to confirm fish absence (i.e. large lake with deep water). An exception was made when fish were present but the water body was too shallow or small to accommodate a gill net (i.e. pools in wet meadows, although sometimes containing fish, were typically too small to gill net). Habitat data were collected at each survey site, including air and water temperature, maximum depth, littoral zone and onshore substrate characteristics, and amount of littoral zone woody debris and aquatic vegetation. Sites were defined as a lake if they were comprised of open water, and were larger than 0.5 hectare. Ponds were distinguished as permanent or temporary using quantitative and qualitative measures such as depth, level of drying and size of watershed feeding the pond. A representative photograph of each site surveyed was taken with a digital camera.

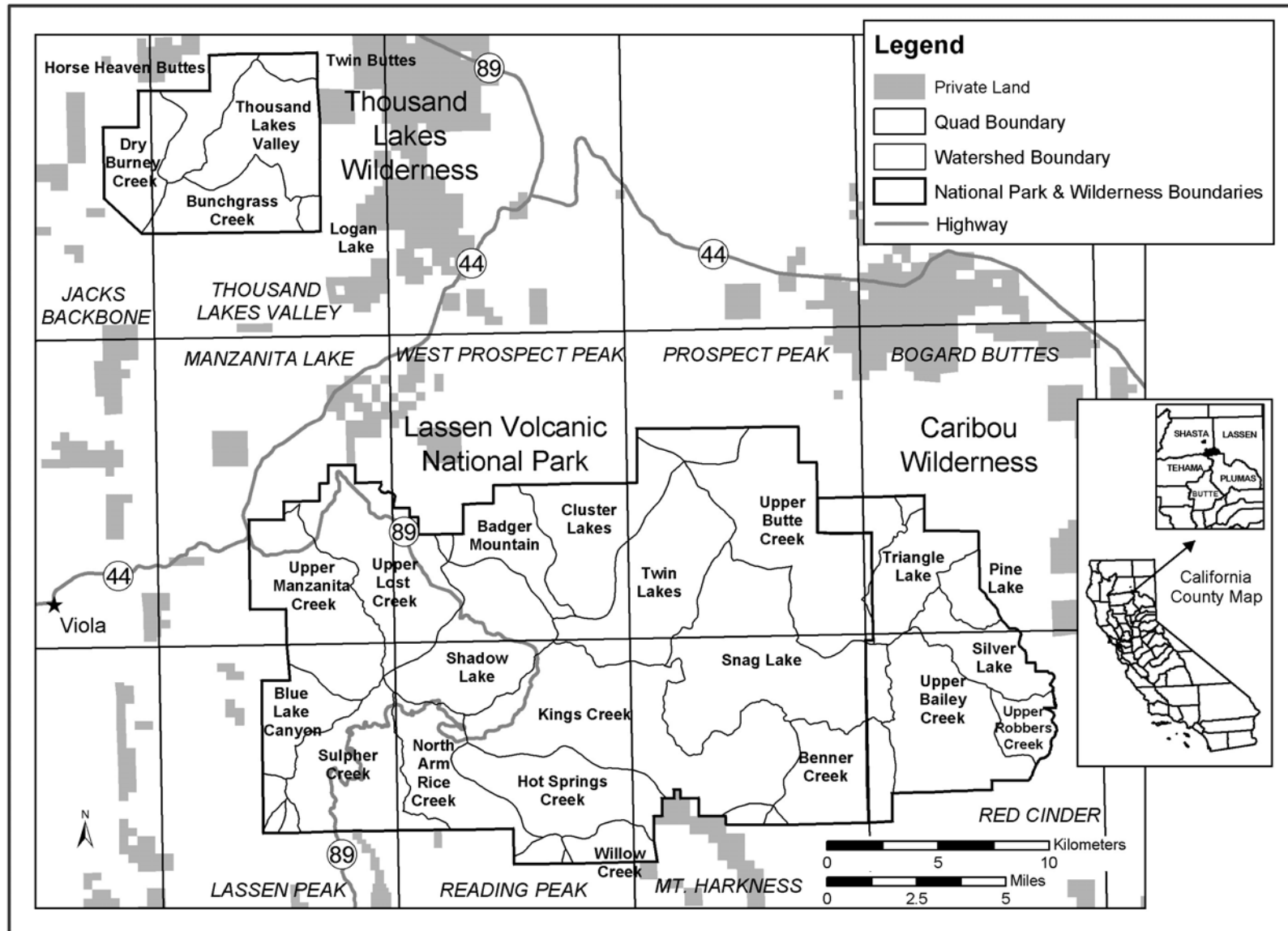
To avoid unintentionally spreading disease between populations of amphibians, all gear and equipment that came in contact with the aquatic habitat during surveys was decontaminated using diluted quaternary ammonia before being transported between

watersheds. For the purposes of this investigation the watershed boundaries used were consistent with the CalWater watershed boundaries (Figure 1).

#### Thousand Lakes and Caribou Wilderness Areas

Thousand Lakes Wilderness was surveyed June 18 through July 4, 2002, and the Caribou Wilderness was surveyed June 11 through September 2, 2002, both by RSL personnel. Karen Pope was the lead biologist at the time, and Don Ashton and Jamie Bettaso led field crews. The surveys were carried out in the same manner as the surveys conducted in LVNP, using a nearly identical protocol and data sheets. All mapped sites in the Thousand Lakes Wilderness were surveyed, plus all unmapped sites encountered incidentally. In the Caribou Wilderness a subset of mapped sites were surveyed, as well as unmapped sites encountered incidentally.

Figure 1. Delineation of CalWater watershed and 7.5' USGS Quad boundaries.



## **Data Analysis**

The field data were entered into an electronic database in Paradox. Data summaries were prepared by querying the Paradox database, and are generally presented in tabular form. For site summaries, we did not include repeat visits, or sites that were visited and determined not to provide the lentic-type habitat targeted by this study. Species presence was determined at a site if any life stage of that species was detected during any visit to the site, including incidental detections outside of the timed visual encounter survey and detections from the first 50 meters of any inlets and outlets present at the site.

Some sites at LVNP were historically stocked with fish, but have since reverted naturally to a fishless condition. This situation lends itself well to a comparison of attributes between lakes that have gone fishless and those that maintain self-sustaining fish populations. Previously compiled stocking records for LVNP were used to generate a list of 42 sites with confirmed fish stocking prior to 1980 (Potts and Schulz 1953; A. Denniston, pers. comm.). Using only the subset of confirmed historically stocked lakes, we divided the lakes into two groups based on fish presence (11 sites) or absence (31 sites). We then used Mann-Whitney U tests to assess whether certain characteristics of the lakes (elevation, number of inlets, perimeter, and area) could be used to differentiate self-sustaining lakes from lakes that naturally reverted to a fishless condition. We ran the analyses separately for all fish species and for only species of trout. Multinomial logistic regression was used to determine the combination of variables best able to predict differences in fish presence at sites historically stocked.

In order to assess the landscape-scale patterns of distribution of amphibians in relation to fish, we applied non-parametric regression techniques to our dataset for LVNP and the two proximal wilderness areas. We used generalized additive models (GAMs) to assess the effects of introduced fish on the presence of amphibians, while controlling for differences in habitat characteristics. We used GAMs for the analyses because they relax the assumption that the relationships between the dependent variable and independent variables are linear. Relaxation of this assumption is accomplished using a nonparametric smoothing function to describe the relationships between the dependent and independent variables. Four models were run for detection/non-detection of long-toed salamander, Pacific treefrog, western toad, and “palatable species”. Palatable species are the amphibians that have been found to be edible by fish (Welsh et al. in review), and include Cascades frog, long-toed salamander and Pacific treefrog. Cascades frogs were too rare for any statistical analyses to be run on them independently. Only one visit per site was used in the analyses, and dry or frozen sites were not included.

We first calculated Pearson correlation coefficients ( $r$ ) for all pairwise combinations of independent variables. In multiple regression, colinearity between predictor variables may confound their independent effects. Site type and water depth had a fairly high correlation value (0.64), so only depth was used in the model to reduce colinearity. We found that none of the amphibian species were highly correlated with each other ( $|r| < 0.3$ ) and, therefore, were comfortable running independent models for each species with sufficient detections to be statistically relevant. The same seven covariates were used in all four of the models. The covariates were fish presence, depth category, elevation,

Julian day, research area (LVNP, Thousand Lakes Wilderness, or Caribou Wilderness), presence of inlets, and location (UTM coordinates).

The strength of the independent variables was determined by evaluating the change in deviance resulting from dropping each variable from the model in the presence of all other variables (test deviance). Analysis of deviance and likelihood ratio tests (based on the binomial distribution) were used to test the significance of each of the independent variables on the probability of frog or salamander presence. Akaike's Information Criterion (AIC) was used to rank each of the variables in the models by order of importance. When assessing AIC values, it is not the absolute size of the AIC value that is important, but the change in the value due to removal of a variable compared to the full-model AIC value. Using the coefficient values for the fish variable from the GAMs, we estimated the change in the odds of finding the different amphibians in the presence versus absence of fish while controlling for the effects of the habitat variables.



## Results

During the 2004 summer field season we conducted 442 site visits at LVNP, and surveyed all mapped sites at least once. These visits were conducted at a total of 365 unique sites, shown in Figure 2, and included 68 second visits, or re-surveys, conducted in the latter portion of the field season. During the 2002 summer field season we conducted 78 site visits in the Thousand Lakes Wilderness, and surveyed all mapped sites once. In the Caribou Wilderness, 455 individual sites were visited. There are 196 mapped sites in the Caribou Wilderness that have not yet been surveyed. In the following subsections, we first present species and/or species group summaries, followed by the results of the statistical analyses.

### Amphibians

Amphibian species detected within LVNP during the 2004 field season include Pacific treefrog (*Hyla regilla*), western toad (*Bufo boreas*), long-toed salamander (*Ambystoma macrodactylum*), Cascades frog (*Rana cascadae*), and rough-skinned newt (*Taricha granulosa*). At least one species of amphibian was detected at 61% of the sites surveyed at LVNP, 49% of the sites surveyed in the Caribou Wilderness, and 89% of the sites surveyed in the Thousand Lakes Wilderness (Table 1). Neither Cascades frog nor rough-skinned newts were detected in either of the proximal wilderness areas.

### Cascades Frog

The Cascades frog was detected at only three of the sites surveyed in LVNP, all located within the Kings Creek watershed, at or near Juniper Lake (Figure 3), and was not detected in either of the proximal wilderness areas surveyed. One adult and one subadult frog were detected at a temporary pond near Juniper Lake, on June 30, and another adult frog was detected at a relatively small, fishless lake located just west of Juniper Lake, on July 1. We revisited the Juniper Lake basin and a number of other sites in the vicinity in the middle of August, and found only one adult Cascades frog, on the western shore of Juniper Lake, where the drainage from Indian Lake enters. Frogs were not detected at either of the small, nameless sites where they had previously been found. No Cascades frog egg masses, larvae, or evidence of breeding was encountered during the course of the surveys.

Figure 2. Locations of all sites surveyed.

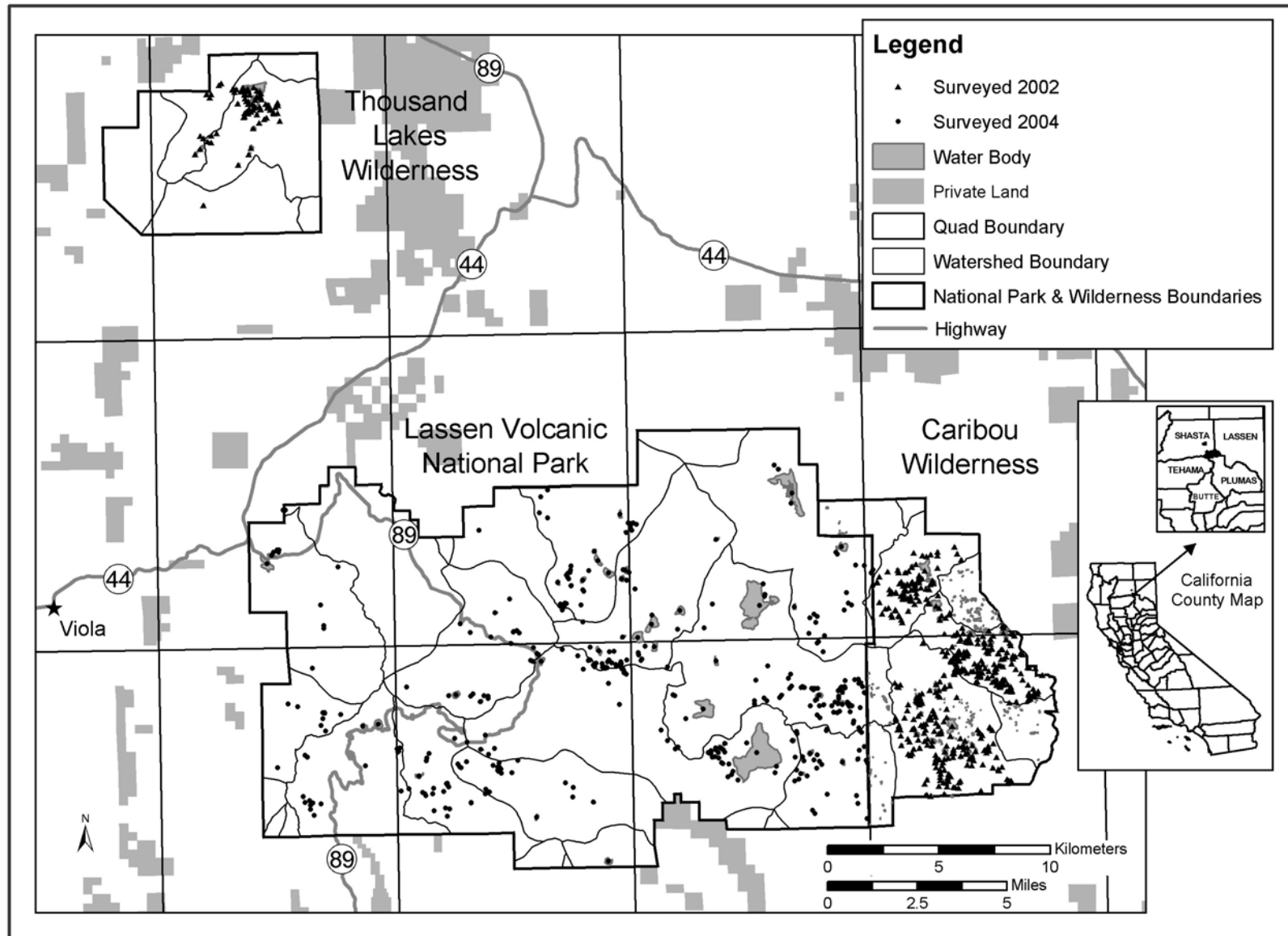
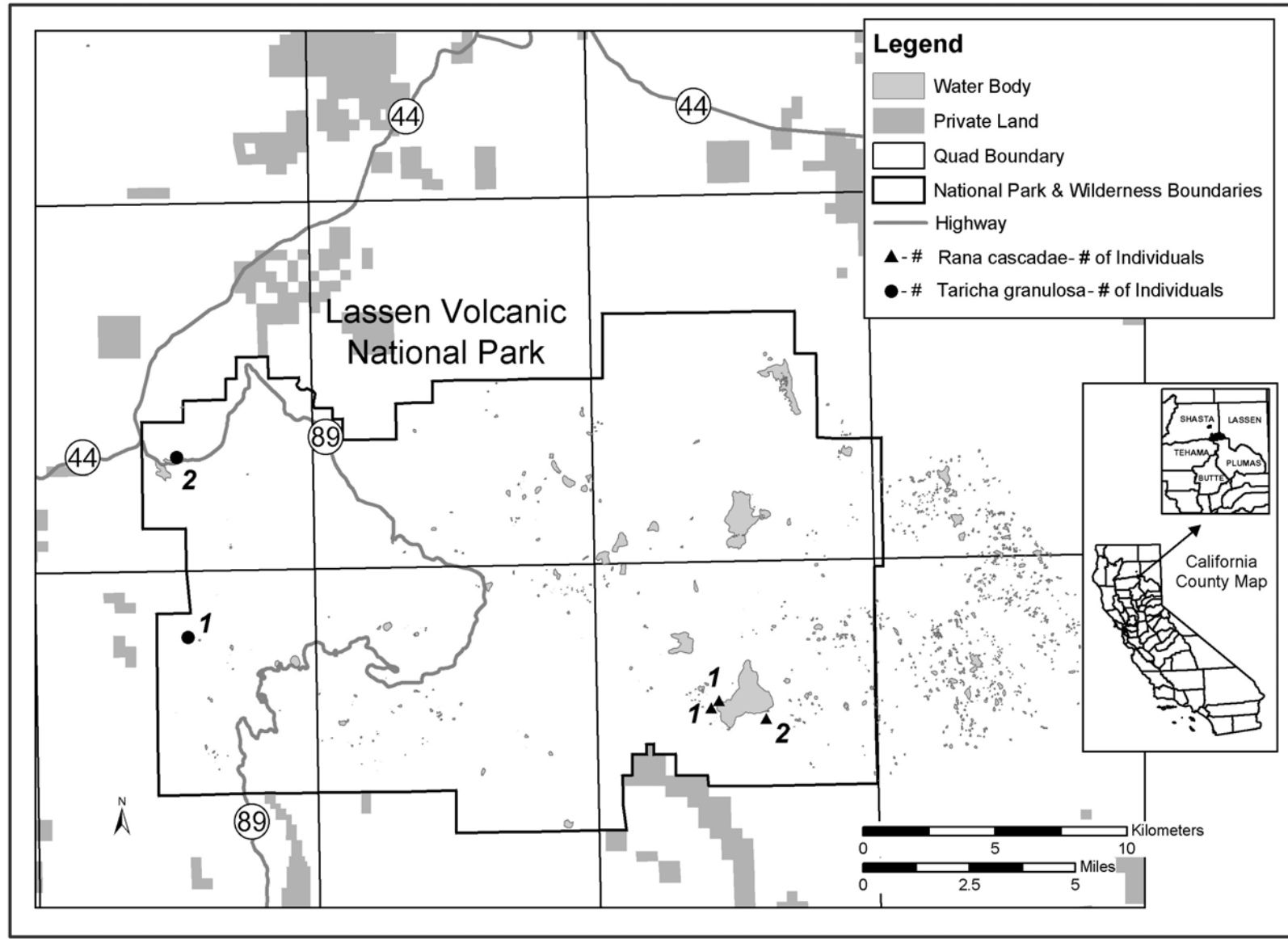


Table 1. Summary of amphibian presence data, showing number and percent of sites where amphibians were detected.

Habitat Type (# of sites)	Number (and Percent) of Sites With					
	<i>Rana cascadae</i>	<i>Taricha granulosa</i>	<i>Bufo boreas</i>	<i>Ambystoma macrodactylum</i>	<i>Hyla regilla</i>	Any Amphibian
Lassen Volcanic National Park						
Lake (57)	2 (4%)	0	12 (21%)	11 (19%)	48 (84%)	51 (90%)
Permanent Pond (94)	0	1 (1%)	6 (6%)	8 (9%)	59 (63%)	60 (64%)
Temporary Pond (173)	1 (1%)	1 (1%)	6 (3%)	13 (8%)	79 (46%)	82 (47%)
Wet Meadow (40)	0	0	5 (13%)	4 (10%)	27 (68%)	29 (73%)
All Sites (365)	3 (1%)	2 (1%)	29 (8%)	36 (10%)	214 (59%)	223 (61%)
Caribou Wilderness						
Lake (53)	0	0	1 (2%)	7 (13%)	29 (55%)	32 (60%)
Permanent Pond (197)	0	0	0	37 (19%)	121 (61%)	127 (64%)
Temporary Pond (195)	0	0	0	16 (8%)	57 (29%)	59 (30%)
Wet Meadow (8)	0	0	0	0	6 (75%)	6 (75%)
Marsh/Bog (2)	0	0	0	0	1 (50%)	1 (50%)
All Sites (455)	0	0	1 (0%)	60 (13%)	214 (47%)	225 (49%)
Thousand Lakes Wilderness						
Lake (11)	0	0	2 (18%)	1 (9%)	11 (100%)	11 (100%)
Permanent Pond (32)	0	0	3 (9%)	9 (28%)	29 (91%)	29 (91%)
Temporary Pond (29)	0	0	2 (7%)	9 (31%)	23 (79%)	24 (83%)
All Sites (72)	0	0	7 (10%)	19 (26%)	63 (88%)	64 (89%)

Figure 3. Sites with detections of Cascades frogs and rough-skinned newts.



Two unidentified anurans were detected at Crag's Lake, in the Upper Manzanita Creek watershed. These two subadult or adult anurans, first observed on June 19, appeared to be of a size that could be either a large treefrog or small ranid frog. The frogs were first observed floating on the surface, in the middle of Crag's Lake. Any attempt to get close resulted in the animals diving deep into the center of the pond, underneath a large boulder, and a positive identification was not obtained (although treefrog egg masses were present at the time). On June 25 park personnel provided us with a photograph of a frog floating on the surface of Crag's Lake, taken by a park volunteer sometime in early June. The consensus of RSL herpetologists is that the animal in the photograph could be either a ranid or a hylid frog, and nothing definite can be said about the image. Also on the 25 of June, we returned to Crag's Lake to search for the elusive frogs. Multiple shoreline searches and a snorkel survey of the pond's bottom conducted on that day failed to detect any frogs (except for the then hatching treefrog egg masses). Finally, the site was resurveyed on August 12, when treefrog larvae and metamorphs were both present. Although two Cascades frogs may have been present at Crag's Lake during the first part of June, repeated attempts to confirm their presence were unsuccessful.

Although located at the southern edge of the species' historic geographic range, the Cascades frog was once common in and around LVNP (Grinnell et al. 1930; CNDDDB 2003; Koo et al. 2004), but by 1991 populations had already shrunk to critically low levels (Fellers and Drost 1993). Surveys of 50 sites in LVNP conducted at that time, including 16 historic Cascades frog localities, resulted in the detection of only two adult frogs, with no other life stage detected. Whether Cascades frog numbers were as low

throughout LVNP in 1991, when Fellers and Drost conducted their surveys, as they are now, is unknown, since surveys conducted at that time were less extensive than ours. However, the situation has clearly not improved since 1991. Recent surveys of Lassen National Forest indicate that the species' current distribution in the Forest is limited to just a few localities, none of which seem to support robust populations (Fellers 1997; Koo et al. 2003). Consequently, the data would suggest the Cascades frog is at immediate risk of extirpation from the Lassen region.

#### Rough-skinned Newt

The rough-skinned newt was detected at two sites in LVNP, and was not detected in either of the proximal wilderness areas (Figure 3). Two subadult newts were detected in the outlet of a temporary pond near Reflection Lake, in the Upper Manzanita Creek watershed, and one adult newt was detected in a permanent pond in Blue Lake Canyon. The sites are located in adjacent watersheds on the western edge of the park, and are both at the upper end of the larger Battle Creek watershed, which flows west to the Sacramento River.

Stebbins (2003) reports that this species occurs chiefly west of the crest of the Cascades Mountains, and in the Lassen region there are no historic occurrences documented from east of the Cascade/Sierra Crest (Koo et al. 2004). Considering that the majority of LVNP and all of the Caribou Wilderness lie east of the Cascade/Sierra crest, most of the area encompassed by this investigation lies outside of the historic range of the rough-skinned

newt. Therefore, it is not surprising that we found them in low numbers only near the western boundary of the Park, on the west side of the Cascade/Sierra crest.

### Western Toad

The western toad was detected at 8% of sites in LVNP, at 10% of sites in the Thousand Lakes Wilderness, and at only one site in the Caribou Wilderness (Figure 4). In LVNP, toads were found at 21% of lakes surveyed, and although 173 temporary ponds were surveyed, toads were detected at only 6 (3%) of them (Table 1). Toads were detected at 13% of wet meadows and 6% of permanent ponds in the Park. In the Thousand Lakes Wilderness, toads were also found in a higher percentage of lakes than other site types surveyed.

Although toads were detected at a slightly higher percentage of sites in the Thousand Lakes Wilderness than in LVNP (Table 1), toads were more abundant at LVNP. The largest number of toads (any life stage) encountered during a single survey at any given site in the Thousand Lakes Wilderness was 45, compared to the 34,007 individuals counted at one site in LVNP (although these surveys were conducted during different years). A total of 75,539 toads were counted in LVNP, and 8 sites had over 1,000 toads, while only 60 toads were detected in all of the Thousand Lakes Wilderness (Table 2). The size and apparent reproductive success of toad populations in LVNP is significant, relative to the much smaller populations detected in the proximal wilderness areas, and in light of the apparent decline of toads from some parts of the west.

Figure 4. Sites with detections of the western toad.

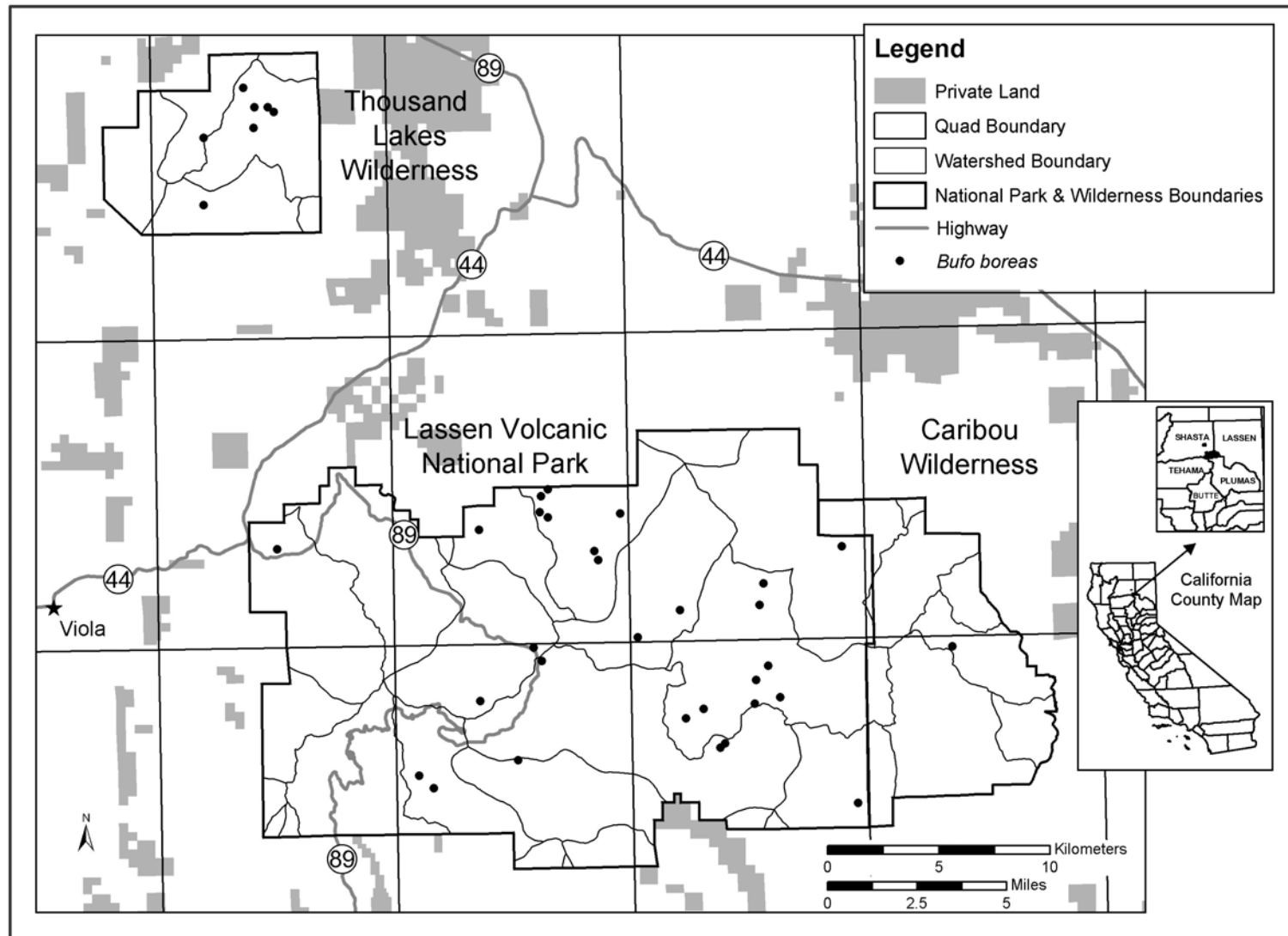


Table 2. Summary of count data for amphibian species commonly encountered.

Research Area (# Sites Surveyed)	Includes Sum of Individuals in All Life Stages				
	# (and %) Sites with ≥10,000 Animals	# (and %) Sites with ≥1,000 Animals	# (and %) Sites with ≥100 Animals	# (and %) Sites with ≥10 Animals	Total # Animals Detected*
Western Toad					
Lassen Volcanic National Park (365)	2 (1%)	8 (2%)	14 (4%)	15 (4%)	75,539
Caribou Wilderness (455)	0	0	0	0	1
Thousand Lakes Wilderness (72)	0	0	0	1 (1%)	60
Long-toed Salamander					
Lassen Volcanic National Park (365)	0	0	0	7 (2%)	426
Caribou Wilderness (455)	0	0	0	14 (3%)	461
Thousand Lakes Wilderness (72)	0	0	2 (3%)	11 (15%)	989
Pacific Treefrog					
Lassen Volcanic National Park (365)	2 (1%)	22 (6%)	78 (21%)	152 (42%)	99,490
Caribou Wilderness (455)	0	2 (0%)	43 (9%)	136 (30%)	22,709
Thousand Lakes Wilderness (72)	0	7 (10%)	48 (67%)	57 (79%)	29,465

\*When a site was surveyed more than once, only the largest number of animals detected during any one visit was used to calculate this value.

### Long-toed salamander

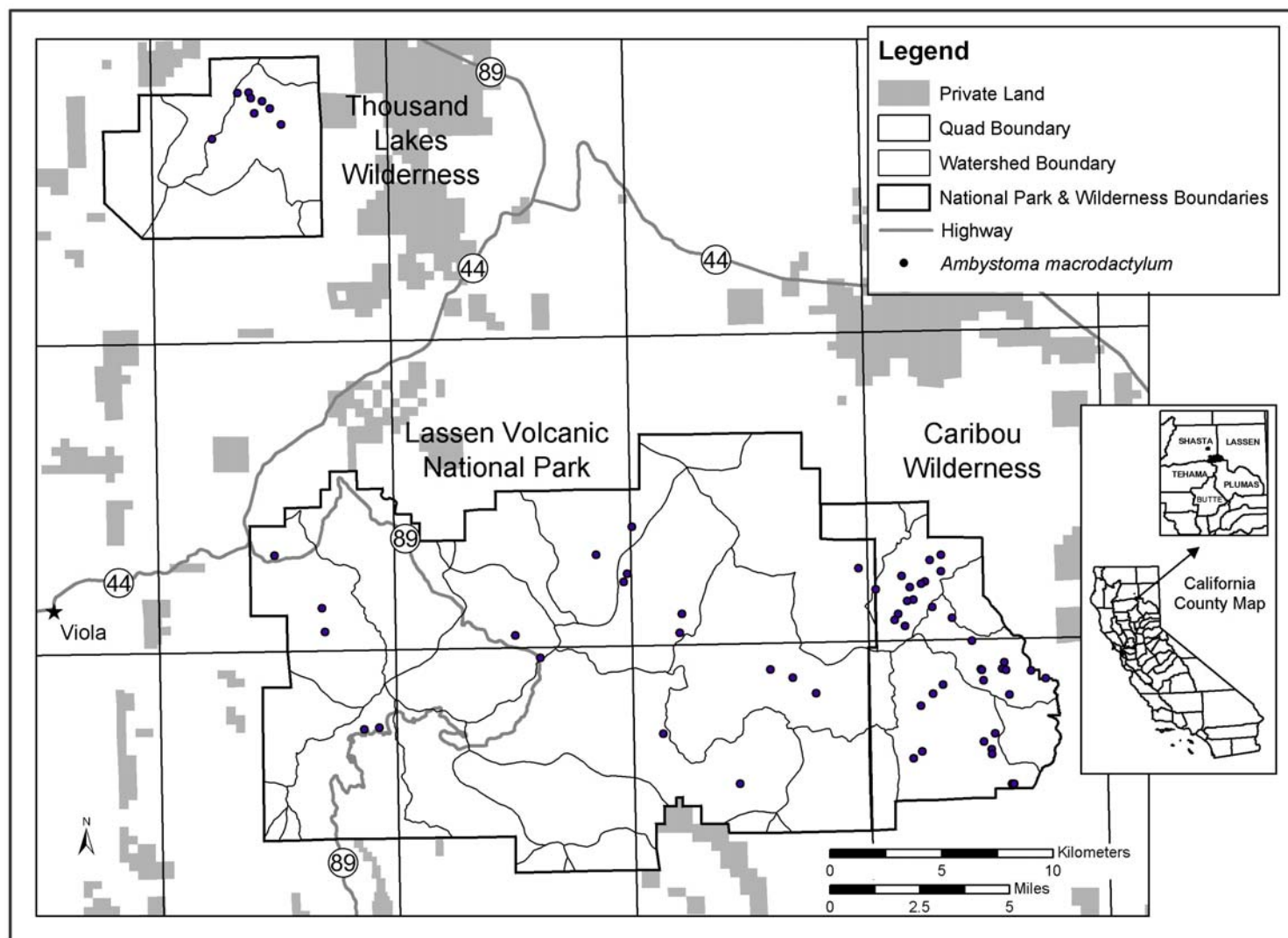
The long-toed salamander was detected at 10% of sites in LVNP, 13% of sites in the Caribou Wilderness, and 26% of sites in the Thousand Lakes Wilderness (Table 1, Figure 5). In both LVNP and the two proximal wilderness areas, salamanders were detected in lakes, permanent ponds, and temporary ponds. In LVNP they were also detected in wet meadows. Salamanders were typically detected in low numbers, and the largest number of individuals of any life stage detected at any site in LVNP was 85. Ten or more individuals were counted at only seven sites in the Park (Table 2). Although the Thousand Lakes Wilderness is substantially smaller than LVNP, and has only a fraction of the total number of survey sites present in the Park, or in the adjacent Caribou Wilderness, over twice as many long-toed salamanders were detected in the Thousand Lakes Wilderness than were detected in LVNP or in the Caribou Wilderness.

During the surveys we conducted at LVNP, we found what seemed to be an unusually high number of dead long-toed salamander larvae at a few survey sites, where no obvious reason for mass mortality could be determined. These dead animals were typically large, well developed larvae, probably nearing metamorphosis. On August 15 we encountered 33 live long-toed salamander larvae, and another 17 that were dead, at the only mapped site in the Badger Mountain watershed (site 10422). One of the dead larvae was collected, and was later checked for the presence of chytrid fungus (*Batrachochytrium dendrobatidis*) by Jess Morgan at UC Berkeley, using PCR analysis. The analysis was negative, and chytrid was determined not to be present in that dead animal. We generally found salamanders at sites distributed around most of LVNP, but they were not detected

at any of the sites surveyed in the North Arm Rice Creek, Benner Creek, or Hot Springs Creek watersheds, all located along the southern boundary of the park. Hot Springs Creek watershed includes the Sifford Lakes, where DiMartini et al. (2000) found both live and dead salamanders. They found salamander larvae or eggs at six of the twelve Sifford Lakes (using different survey techniques than ours), and on July 19, 2000 they encountered a few live, and many recently dead, larval long-toed salamanders at one of the Sifford Lakes. Although we surveyed all twelve of the Sifford Lakes twice, long-toed salamanders were not detected during any of our surveys. The situation with long-toed salamander in and around LVNP clearly calls for further investigation.

Of the 115 sites in the three study areas where long-toed salamanders were detected, only two contained fish. The two sites where salamanders did co-occur with fish are both wet meadows, where fish are typically present in areas with seasonal connectivity to streams that support fish, but fishless habitat also occurs.

Figure 5. Sites with detections of the long-toed salamander.



### Pacific Treefrog

The Pacific treefrogs was the most commonly encountered amphibian species in this investigation. Treefrogs were detected at 61% of sites in LVNP, 49% of sites in the Caribou Wilderness, and 89% of sites in the Thousand Lakes Wilderness (Table 1, Figure 6). They were detected in all site types surveyed, and were generally more often found associated with permanent water, such as in lakes and permanent ponds. Wet meadows, which may have permanent or temporary water, also often supported Pacific treefrogs. Like the long-toed salamander, treefrogs were more common in the Thousand Lakes Wilderness than elsewhere.

### **Reptiles**

While conducting visual encounter surveys for amphibians we also counted reptiles, the majority of which were garter snakes (*Thamnophis* spp.). Garter snakes are known to be important predators of amphibians in environments such as LVNP (Jennings et al. 1992; Matthews et al. 2002), and are one of the target species for the visual encounter survey. Other reptile species detected at LVNP include the northern and southern alligator lizards (*Elgaria coerulea* and *E. multicarinata*, respectively), the sagebrush lizard (*Sceloporus graciosus*) and the western fence lizard (*S. occidentalis*). One rubber boa (*Charina bottae*) was encountered incidentally in an upland habitat in Blue Lake Canyon, at the western boundary of LVNP.

### Garter Snakes

Garter snakes were detected at 26% of sites at LVNP, 19% of sites in the Caribou Wilderness and at 22% of sites in the Thousand Lakes Wilderness (Table 3). Garter snakes were detected at lakes, permanent ponds, temporary ponds and wet meadows. Two species of garter snake were detected at LVNP and in the Caribou Wilderness, the western terrestrial garter snake (*Thamnophis elegans*), and the common garter snake (*T. sirtalis*). *T. sirtalis* was more common than *T. elegans* in both areas, and only *T. sirtalis* was detected in the Thousand Lakes Wilderness.

Figure 6. Sites with detections of the Pacific treefrog.

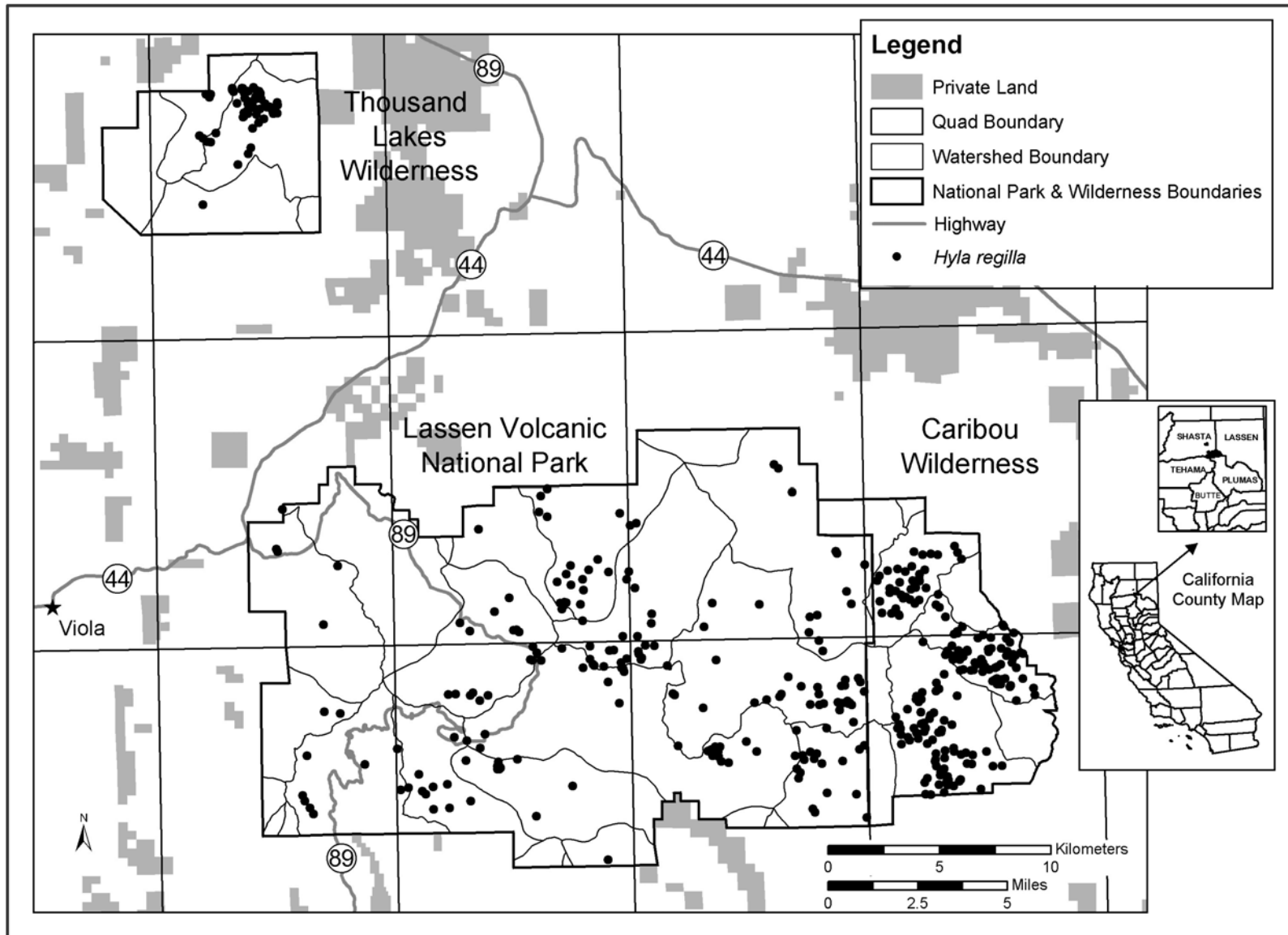


Table 3. Summary of garter snake presence data, showing number and percent of sites where species were detected.

<b>Habitat Type (# of sites)</b>	<b><i>Thamnophis sirtalis</i></b>	<b><i>Thamnophis elegans</i></b>	<b><i>Thamnophis sp.</i></b>	<b><i>Thamnophis spp.</i></b>
<b>Lassen Volcanic National Park</b>				
Lake (57)	13 (23%)	5 (9%)	30 (53%)	35 (61%)
Permanent Pond (94)	7 (7%)	0	23 (24%)	26 (28%)
Temporary Pond (173)	11 (6%)	1 (1%)	15 (9%)	25 (14%)
Wet Meadow (40)	4 (10%)	0	8 (20%)	10 (25%)
All Sites (365)	35 (10%)	6 (2%)	76 (21%)	96 (26%)
<b>Caribou Wilderness</b>				
Lake (53)	13 (25%)	9 (17%)	4 (8%)	22 (42%)
Permanent Pond (197)	24 (12%)	10 (5%)	11 (6%)	41 (21%)
Temporary Pond (195)	17 (9%)	2 (1%)	3 (2%)	21 (11%)
Wet Meadow (8)	4 (50%)	0	0	4 (50%)
All Sites (455)	58 (13%)	21 (5%)	18 (4%)	88 (19%)
<b>Thousand Lakes Wilderness</b>				
Lake (11)	5 (45%)	0	1 (9%)	6 (55%)
Permanent Pond (32)	3 (9%)	0	1 (3%)	4 (13%)
Temporary Pond (29)	6 (21%)	0	0	6 (21%)
All Sites (72)	14 (19%)	0	2 (3%)	16 (22%)

## Fishes

Fish species detected at LVNP during the 2004 field season include brook trout (*Salvelinus fontinalis*), brown trout (*Salmo trutta*), rainbow trout (*Oncorhynchus mykiss*), tui chub (*Gila bicolor*), golden shiner (*Notemigonus crysoleucas*), fathead minnow (*Pimephales promelas*), speckled dace (*Rhinichthys osculus*), Lahontan redbreast (*Richardsonius egregius*) and Tahoe sucker (*Catostomus tahoensis*). The results of fish surveys conducted at LVNP are summarized in Table 4. Of the nine species detected in the Park, five are native to parts of California, including rainbow trout, tui chub, speckled dace, Lahontan redbreast and the Tahoe sucker. However, the vast majority of the aquatic habitat in LVNP was probably historically devoid of fish due to natural fish blockages, such as cascades, that prevented fish from naturally colonizing high elevation waters in the Sierra Nevada and Cascade mountains. Previous authors have included most of LVNP within a large portion of the Sierra Nevada presumed to be historically fishless (Moyle and Randall 1998). That being said, it is possible that Manzanita Lake and Butte Lake may have had native fish populations, due to their connectivity with trout streams. Generally speaking, trout occurring in LVNP are the result of historic stocking efforts, and other species are naturalized “bait fish” released over the years by anglers in pursuit of trout.

Fish are distributed widely throughout the three study areas (Table 5, Figure 7). At LVNP, we detected fish at 23 survey sites, primarily in lakes, permanent ponds and wet meadows. About half of these sites contain only trout (Table 4). Eleven of the 42 sites that were documented as historically stocked still support fish. Four of those sites have

only non-trout species, so actually only eight of the historically stocked sites now have reproducing trout populations. Winterkill and lack of suitable spawning habitat likely resulted in extirpation of trout from the other 34 sites historically stocked. Non-trout species may have persisted in some lakes where trout have been extirpated due to less restrictive spawning requirements.

Fish were also present at twelve sites in LVNP where we could not confirm historic stocking. Some of these sites may have been stocked, although without record. Others were likely colonized by fish traveling from stocked lakes via connecting streams. Presumably, all of the fish that we detected are the result of self-sustaining populations, since fish stocking in the Park has not occurred since 1980. Sites that support fish but lack spawning habitat probably have connectivity during some or all of the year with spawning habitat at another location (i.e. in-stream spawning habitat located up or downstream from a lake without spawning habitat could be the source of fish found in the lake). It should be noted that there are additional places in LVNP where stream habitat supports fish, in streams that were not surveyed as part of this lentic habitat study.

Table 4. Detailed summary of fish survey results at Lassen Volcanic National Park.

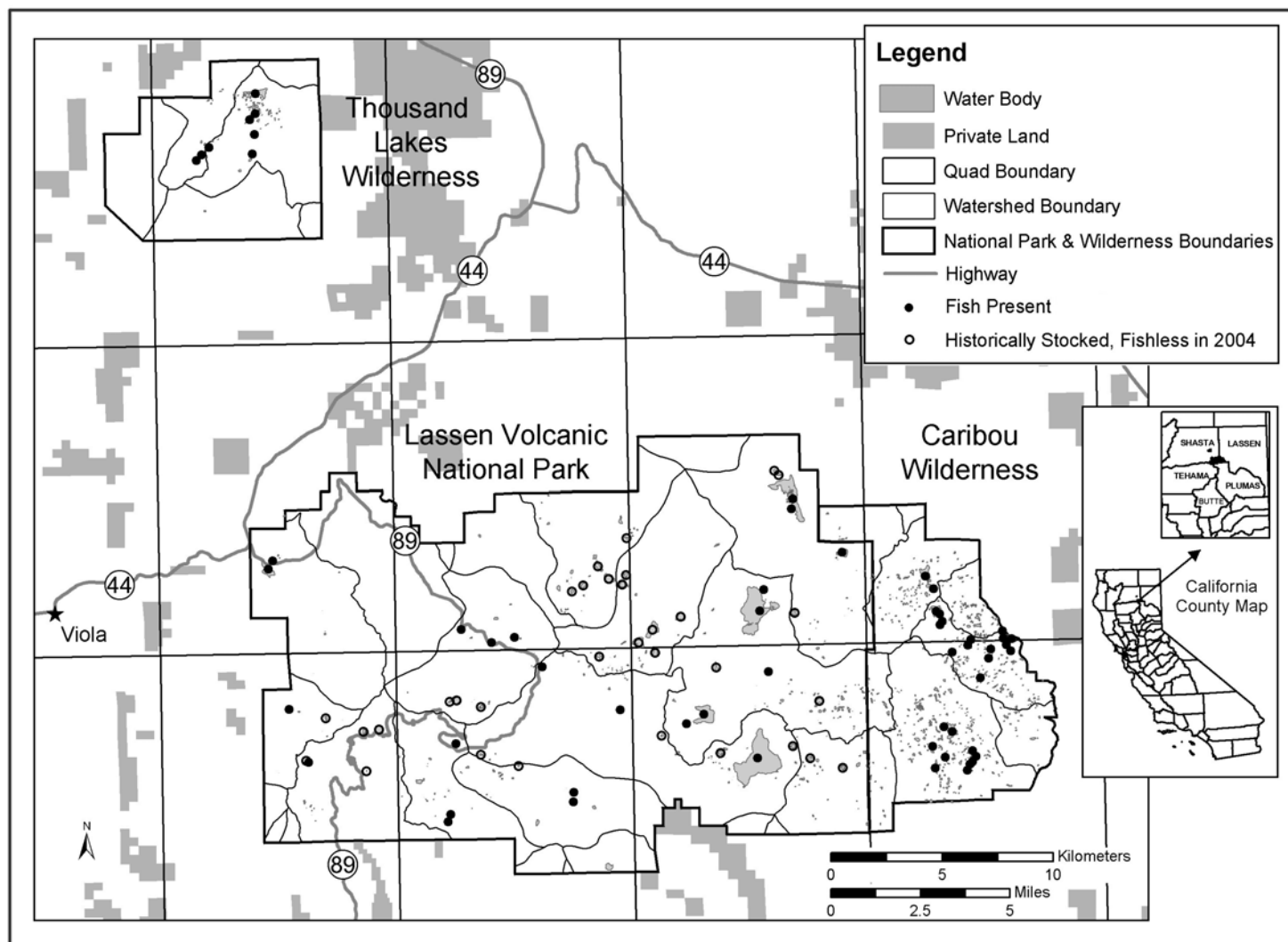
SiteId	Site Name	SiteType	Brook Trout	Brown Trout	Rainbow Trout	Un-ID'd Trout	Tui Chub	Golden Shiner	Fathead Minnow	Speckled Dace	Lahontan Redside	Tahoe Sucker	Un-ID'd Fish
Blue Lake Canyon Watershed													
10956	-	perm. pond			35								
Hot Springs Creek Watershed													
11373	Dream Lk.	perm. pond	1	1									
11373.01	-	temp. pond				P							
Kings Creek Watershed													
10793	Summit Lk.	lake	12										
10912.01	Grassy Swale Mdw.	wet meadow											P
11144	Juniper Lk.	lake					P				6		
11211.01	Upper Mdw.	wet meadow				P							
North Arm Rice Creek Watershed													
11421.01	Upper Twin Mdw.	wet meadow				P							
11421.02	Lower Twin Mdw.	wet meadow				P							
Shadow Lake Watershed													
10648	Hat Lk.	perm. pond	11										
10694.01	-	wet meadow				P							
10694.03	-	wet meadow				P							
Snag Lake Watershed													
10510	Snag Lk.	lake			7		1			P		3	
10510.01	-	temp. pond											P
10893.01	Cameron Mdw.	wet meadow				P							
11026	Horseshoe Lk.	lake		6			21						
11026.01	-	wet meadow											P
Sulphur Creek Watershed													
11221	Ridge lakes	lake	5										
Upper Butte Creek Watershed													
10406	Butte Lk.	lake			2		30			P	41	68	
10412	-	perm. pond								P			
10445	Widow Lk.	lake					25			P			
Upper Manzanita Creek Watershed													
10437	Reflection Lk.	lake						413	1				
10438	Manzanita Lk.	lake	P	6	4			P		P			

P = present; numbers represent fish captured in gill net

Table 5. Summary of fish and fairy shrimp presence data, showing number and percent of sites where each was detected.

<b>Habitat Type (# of sites)</b>	<b>Number (and Percent) of Sites With</b>	
	<b>Fish Presence</b>	<b>Fairy Shrimp Detections</b>
<b>Lassen Volcanic National Park</b>		
Lake (57)	9 (16%)	2 (4%)
Permanent Pond (94)	4 (4%)	11 (12%)
Temporary Pond (173)	2 (1%)	37 (21%)
Wet Meadow (40)	8 (6%)	4 (10%)
All Sites (365)	23 (6%)	54 (15%)
<b>Caribou Wilderness</b>		
Lake (53)	22 (42%)	9 (17%)
Permanent Pond (197)	4 (2%)	108 (55%)
Temporary Pond (195)	0	76 (39%)
Wet Meadow (8)	1 (13%)	2 (25%)
All Sites (455)	27 (6%)	195 (43%)
<b>Thousand Lakes Wilderness</b>		
Lake (11)	8 (73%)	3 (27%)
Permanent Pond (32)	0	24 (75%)
Temporary Pond (29)	0	25 (86%)
All Sites (72)	8 (11%)	52 (72%)

Figure 7. Sites with fish presence, and historically stocked sites now fishless.



## Fairy Shrimp

The only species of fairy shrimp detected at LVNP was *Streptocephalus sealii*. This species is known from a number of sites in the Cascades-Sierra Nevada region (Eriksen and Belk 1999). Field crews at LVNP were trained to identify adult male specimens of *S. sealii* at the beginning of the season, and were instructed to make a collection if an adult male specimen was determined not to be *S. sealii*, to be identified later. A number of populations of fairy shrimp were encountered and not identified to species, either because the animals were immature or because no males were captured. Field crews working in the proximal wilderness areas in 2002 were not trained in fairy shrimp identification, and did not differentiate between different species that may have been encountered in the field. However, a number of collections were made, and subsequently examined in the laboratory. All specimens collected from the Thousand Lakes Wilderness, and the majority of specimens collected from the Caribou Wilderness, are *S. sealii*. In the Caribou Wilderness, specimens of a second species, *Branchinecta coloradensis*, were collected from two separate locations. *B. coloradensis* is widespread in western North America (Belk and Rogers 2002).

Because fairy shrimp were not always identified to the species level, the following data summary refers to all fairy shrimp, whether species was determined or not, as one group, classified simply as fairy shrimp. Fairy shrimp were detected at 15% of sites in LVNP, 43% of sites in the Caribou Wilderness and at 72% of sites surveyed in the Thousand Lakes Wilderness (Table 5). They were present in a variety of site types including lakes, permanent ponds, temporary ponds and wet meadows, but were never found co-occurring

with fish. The introduction of fish may have had a profound impact on the distribution of fairy shrimp (and other invertebrates) in the study area. Invertebrates collected at LVNP during the 2004 field season by our field crews are currently being keyed out at southern Oregon University, under the supervision of Dr. Michael Parker. The results of that component of this study will be reported separately, and may shed more light on the impact that fish have had on invertebrate communities at LVNP.

## **Data Analysis**

### Historically Stocked Lakes Analysis

In our analysis of historically stocked lakes, the number of inlets, size, and elevation of water bodies all seem to affect the self-sustainability of fish populations in LVNP. The number of inlets was significantly greater in lakes that continue to support fish, compared to those that have gone fishless (Mann-Whiney U Test,  $p=0.0002$ ). This relationship was also significant when the response variable was trout presence, as opposed to presence of any fish species. The number of inlets at a lake or pond reflects its degree of connectivity with adjacent aquatic habitats, and spawning habitat at the mouth of an inlet, or in the inlet itself, may be better than that available in the otherwise lentic water body. Inlets also may carry nutrients and even invertebrates, or fish food, into the water bodies. More than any other variable we looked at, the presence of inlets appears to be associated with the self-sustainability of fish populations at LVNP.

Of the historically stocked sites in LVNP, the elevation of sites that continue to support fish is lower than at the fishless sites ( $p=0.009$ ), and the relationship with elevation

remained significant when the response of trout alone was evaluated. A similar investigation in the southern Sierra Nevada found that trout failed to reproduce above 3520 meters (Armstrong and Knapp 2004). The highest elevation of the historically stocked lakes in LVNP is 2,492 meters, but the more northerly latitude of LVNP influences snow pack, and snow often remains around some of the higher lakes until late July or early August (Schaffer 1981; J. Stead, personal observation). The elevation at which lakes would be unable to support fish, regardless of other characteristics likely to influence their growing season (i.e. aspect, depth, etc.) may be higher in LVNP than the elevation of any of the lakes there, but elevation, and variables associated with elevation (temperature, length of growing season, etc.), have likely played a role in the extirpation of some fish populations in LVNP. The size of water bodies, when measured as perimeter or area, was also greater at sites that continue to support fish (perimeter,  $p=0.04$ ; area,  $p=0.038$ ), although neither relationship was significant when the alternate response variable, trout only, was evaluated. One of the mechanisms by which fish have likely been extirpated from sites is winterkill, which is less likely to occur in larger, deeper lakes.

Based on multinomial logistic regression of the aforementioned variables, the model best able to predict which lakes would revert to a fishless condition included only the number of inlets and perimeter. Utilizing those two measurements alone, the regression model was able to correctly classify the current status (extirpated/extant) of fish populations in 88% of the historically stocked lakes. The classification of fishless lakes was even more accurate than lakes with fish, and the model correctly classified 30 of 31 fishless lakes.

Based on the analysis of deviance, the number of inlets was the more significant variable in the model, but the model was stronger when perimeter was included than it was when number of inlets alone was used. When elevation or area were included in the multinomial regression, with number of inlets, quasi-complete separation occurred and the maximum likelihood routine did not converge, likely due to the small number of lakes in the analysis (particularly in the fish present group, where  $n=11$ ). Perhaps a larger sample size would allow for the inclusion of elevation in such a model, but based on the LVNP data, our best model for predicting current fish status in historically stocked lakes includes only the number of inlets and perimeter.

### Regional Analysis

Results of the logistic regression models suggest that populations of long-toed salamanders and Pacific treefrogs are less likely to be found in water bodies supporting fish (Table 6). On the other hand, western toads appeared to be unaffected by fish presence. Cascades frogs could not be analyzed due to the low number of positive occurrences; however, they were included in the palatable species group, which was found to have a significant negative relationship with fish. Julian day was a significant predictor variable important in predicting the probability of finding amphibians in all four models. Generally, the effect of day on detecting amphibians was negative prior to about the first week in July, and was positive after that week, although the positive effect of day generally began to diminish sometime in August. The exception was western toad, for which the effect of day on detecting the species had its greatest positive effect in the beginning of July, and receded to the negative both prior to, and after that time. The most

important predictor of Pacific treefrog presence was water depth, where treefrogs were positively associated with water more than one meter deep, followed by elevation (a negative association). The most important predictor variable of western toad presence was number of inlets.

After controlling for the effects of habitat, long-toed salamanders were 9.3 times more likely to be found in fishless than fish containing water bodies (odds ratio, approximate 95% confidence limits [CL]: 4.5-14.1). Pacific treefrogs were 3.2 times (CL: 1.2-5.2) more likely to be found in fishless than fish containing water bodies, and when palatable species were considered together as one group they were 4.3 times (CL: 2.3-6.4) more likely to be found in the fishless water bodies. These results agree with results from a similar analysis conducted on our Klamath-Siskiyou region dataset, where long-toed salamanders, Pacific treefrogs and Cascades frogs were all more likely to be found in fishless than fish containing water bodies (Welsh et al. in review).

Table 6. Summary of binomial amphibian presence models.

Analysis of deviance table showing the statistical significance (P value) and Akaike Information Criteria (AIC) of the independent variables for the 4 logistic regression models (N = 799) assessing the probability of finding amphibians at a site.

Dependent variable	Model AIC	Model covariates	Model		Test		P value	AIC	$\Delta$ AIC*	Direction
			Dev	df	Dev	df				
<i>A. macrodactylum</i>	586	Day	556	778	19	4	0.0004	598	12	+
		Fish presence	550	776	13	1	0.0002	597	11	-
		Location (UTM)	563	784	26	9	0.002	594	8	
		Number inlets	539	776	1	1	0.24	585	-1	
		Depth	541	777	3	2	0.18	585	-1	
		Rsrch. area	539	777	2	2	0.35	584	-2	
		Elevation	540	780	3	5	0.65	579	-7	
<i>H. regilla</i>	1018	Depth	987	776	18	2	0.0001	1033	15	+
		Elevation	988	779	20	5	0.001	1028	10	-
		Fish presence	979	775	11	1	0.001	1027	9	-
		Day	980	778	12	4	0.02	1023	5	+
		Location (UTM)	986	783	18	9	0.04	1018	0	
		Rsrch. area	968	776	0.5	2	0.77	1014	-4	
		Number inlets	969	775	1	1	0.39	1017	-1	
<i>B. boreas</i>	226	Number inlets	184	776	7	1	0.008	231	5	+
		Day	188	778	11	3	0.01	230	4	+July1
		Depth	181	777	5	2	0.11	226	0	
		Elevation	187	779	10	5	0.63	226	0	
		Fish presence	177	776	0.2	1	0.66	224	-2	
		Location (UTM)	194	784	17	9	0.06	224	-2	
		Rsrch. area	178	777	1	2	0.57	223	-3	
"Palatable Species" <i>A. macrodactylum</i> or <i>H. regilla</i> or <i>R. cascadae</i>	998	Depth	972	776	25	2	0.000004	1018	20	+
		Fish presence	964	775	16	1	0.00006	1012	14	-
		Elevation	966	779	19	5	0.002	1007	9	-
		Day	961	778	14	4	0.006	1004	6	+
		Number inlets	952	775	4	1	0.048	1000	2	+
		Location (UTM)	966	783	18	9	0.04	997	-1	
		Rsrch. area	947	776	1	2	0.7	993	-5	

\*Change in AIC from the complete model less the covariate. Negative values suggest model is worse with the covariate than without.

## Discussion

Fish stocking has not occurred in LVNP since prior to 1980, but nonnative fish are still present at numerous locations in the Park. The majority of lakes and ponds where fish were documented as being introduced have since naturally reverted back to a fishless condition. However, additional lentic habitats now have established populations of fish likely due to undocumented introductions of fish and movement between connected waters. Although winterkill and a lack of sufficient, suitable spawning habitat were likely responsible for a number of extirpations that have occurred since stocking was halted, the locations in LVNP where fish persist today represent self-sustaining populations that have the potential to persist into perpetuity. Gill netting can be successful in eradicating trout from smaller, shallow lakes with limited connectivity and spawning habitat (Knapp and Matthews 1998), but is likely to be very difficult in all but a few lakes in LVNP. The presence of minnows (Family: Cyprinidae) and the Tahoe sucker further complicates the situation because these species may be more difficult to capture in gill nets and, to our knowledge, a gill net eradication has not been attempted.

The results of this investigation indicate that introduced fish have had a significant impact on native fauna at LVNP, including amphibians and fairy shrimp. Fish have almost certainly affected the distribution and reduced the abundance of palatable amphibian species. Similar to other recent studies conducted in wilderness areas of California (Matthews et al. 2001, Welsh et al. in review), we found Pacific treefrogs and long-toed salamanders to be negatively correlated with introduced fish. In contrast to other studies, we found one species, the Cascades frog, is now so rare in the Lassen

region that we cannot even use statistical techniques to assess its distribution in relation to fish. Fairy shrimp, which can be locally abundant and are likely an important prey item for numerous taxa, can use a variety of habitat types, including lakes, but were simply never found co-occurring with fish. The effect of fish on some other aquatic invertebrates in the system may be just as pronounced, and we hope that the invertebrate component of this study, still in progress, will bring that issue to light. Amphibians and aquatic invertebrates are an important part of the food chain in habitats such as LVNP, and changes in the distribution and abundance of these groups could affect terrestrial vertebrates in higher trophic levels.

That being said, we do not believe that fish are the most significant driver of amphibian declines (particularly Cascades frog) that have been observed in the Lassen region. One observation leading to this conclusion is based on the status of Cascades frog in the Klamath-Siskiyou region of northern California. Surveys conducted there revealed that Cascades frog larvae were 3.7 times more likely to be found in fishless than fish containing waters (Welsh et al. in review). Fish are having an effect on Klamath-Siskiyou amphibians, similar to the fish effect observed in the Lassen region. However, Cascades frog was observed at roughly 50 percent of the sites surveyed there, as opposed to the less than 1 percent of sites surveyed in the Lassen region where Cascades frog was detected. If fish were the main driver behind Cascades frog declines in the Lassen region, we would expect a similar response to fish introductions in the Klamath-Siskiyou region.

Also in the Klamath-Siskiyou region, long-toed salamanders were found to be almost twelve times more sensitive to fish than Cascades frog (Welsh et al. in press), and long-toed salamanders were also highly sensitive to fish in the Lassen region, based on the models presented in this report. If fish were the main driver behind declines in the Lassen region, then we would expect long-toed salamanders to have experienced a decline at least as severe as Cascades frog. Although observations of dead salamanders, apparent extirpations, and generally low numbers of long-toed salamanders at LVNP may be cause for concern, salamanders were found at over 100 sites in the Lassen region, and are apparently doing far better than Cascades frog, which was observed at only three sites in the entire region.

Additionally, the order of events does not point strongly to nonnative fish as the main driver of Lassen declines. Historically, the Cascades frog was regionally abundant (Grinnell et al. 1930; CNDDDB 2003; Koo et al. 2004), and apparently remained abundant at some locations at least through 1974, when 44 Cascades frogs were collected from Dersch Meadows in one day (Snow 1974, in Fellers and Drost 1993). Fish stocking records for LVNP date back to 1928, although there is much anecdotal evidence that haphazard stocking by various backcountry users began years prior to 1928, and continued through the 1970's. In 1968 a total phase out of fish stocking in LVNP was begun, and stocking was immediately ceased at all but 17 lakes, with an average of two additional lakes dropped per year until 1980, when all stocking had been terminated within the Park (A. Denniston, pers. comm.). Therefore, we know that Cascades frog continued to be abundant through many decades of fish stocking. The recent rarity of

Cascades frog in LVNP was first noticed in 1989 (Fellers and Drost 1993), ten years after all fish stocking in the Park had ceased. In 1974 frogs were still abundant, at least at some localities in the Park, and stocking had been greatly reduced from historic intensities for over six years. Apparently Cascades frog was doing relatively well in LVNP through decades of regular fish stocking, but declined drastically shortly after stocking was halted. It appears that some other stressor that postdates fish stocking must have helped drive declines of Cascades frog in LVNP.

One question to consider is why Cascades frog populations have not recovered at some of the now fishless lakes, since their decline in the 1970's and/or 1980's. Recent genetic work has shown that gene flow between populations of Cascades frog separated by more than 10 kilometers is low, and migrations of more than a few kilometers are probably rare (Monsen and Blouin 2004). This conclusion is supported by a recent two-year movement study of 1,255 marked Cascades frogs in the Trinity Alps Wilderness. In that study, which focused on within-basin movement (although out-of-basin movements by three individuals were documented), the average movement of an adult frog captured more than once was 426 meters (J. Garwood, unpublished data). Although one movement of almost two kilometers was recorded, only 20 frogs moved more than one kilometer, and the majority of movements were fairly localized. We know that low numbers of Cascades frogs have been present in and around LVNP since the initial decline, and there is ample, suitable, fishless habitat within close proximity to the few animals that have been observed in the Park since 1990. However, frogs have not been successful in repopulating those habitats. In Oregon, researchers noted that recolonization of an

extirpated population of Cascades frog from one site took 12 years, despite the fact that there was a source population two kilometers away (Blaustein et al. 1994a). It may be that extremely low numbers of frogs, combined with relatively localized movements have prevented Cascades frogs from recolonizing extirpated habitats. However, it is also possible that the factors responsible for the original decline continue to affect the Lassen region, thereby reducing the survivorship of the animals, and limitations inherent in the frogs' movement patterns are not a major factor limiting their recovery.

Unfortunately, we do not believe that our findings answer the question as to what factors, besides, or in addition to fish, have driven the decline of Cascades frog in the Lassen region. A number of hypotheses are currently being investigated throughout the western USA. In California, correlations between upwind agricultural land use, pesticide drift, and amphibian declines suggest that chemicals and pollutants are carried by wind from California's Central Valley into the mountains, affecting amphibians as they are deposited (Davidson et al. 2002; Davidson 2004). This may explain why the Cascades frog is doing much better in the Klamath-Siskiyou region, where prevailing winds come off the ocean, directly into the mountains. The correlations between upwind agricultural chemical application and amphibian declines are supported by direct measurements of pesticides present in precipitation and water in the Sierra Nevada (Zabik and Seiber 1993; Aston and Seiber 1997; McConnell et al. 1998), and by direct measurements of pesticide residue in amphibian tissues in the Sierra Nevada (Fellers et al. 2004), and in LVNP (Datta et al. 1998; Sparling et al. 2001). Some of the evidence suggests that the Lassen region may be less affected by pesticide drift than the central and southern Sierra Nevada,

but there are still far more questions than there are answers regarding the effects of pesticides and other airborne contaminants in the mountains of California. Authors of a recent study that found evidence for nutrient enrichment of high-elevation lakes in the Sierra Nevada suggest that a large number of these lakes may be experiencing mild eutrophication (Sickman et al. 2003). They cite increased levels of atmospheric nitrogen and phosphorous, resulting from local sources such as pesticides and phosphorous rich dust and soil aerially transported from the Central Valley, as well as global sources, as the likely cause of this trend. Although difficult to document, these types of broad environmental changes could be directly, or indirectly, responsible for declining amphibian populations in the Lassen region.

Disease also may have played a role in the decline of Cascades frog from the Lassen region, although we are aware of no direct evidence of such. Diseases, particularly fungal infections, have been implicated in amphibian declines worldwide (Blaustein et al. 1994; Berger et al. 1998; Bosch et al. 2001). A chytrid fungus, *Batrachochytrium dendrobatidis*, can cause mortality in some amphibian species, and infects the mountain yellow-legged frog (*Rana muscosa*) and other Sierra Nevada species (Fellers et al. 2001). Chytrid fungus has been detected in some rapidly declining populations of mountain yellow-legged frog in the Sierra Nevada (R. Knapp, pers. comm.). Although we are not aware of a positive detection of chytrid fungus in Cascades frog, the relatively sudden decline in the Lassen region does fit with the disease hypothesis.

The observed decline of Cascades frog in the Lassen region may be the result of multiple factors acting together, the impacts of which could be amplified by synergistic relationships that may exist between stressors. For example, limb deformities in *Rana sylvatica*, which often result in a frog more susceptible to predation than its non-deformed counterpart, are caused by trematode infections (Kiesecker 2002). In Pennsylvania, Kiesecker (2002) found that deformities were much more common at sites affected by agricultural runoff. Laboratory experiments suggested that pesticides in the runoff might compromise the frog's immune system, making them more susceptible to trematode infections. Complex relationships between natural and anthropogenic factors, which can be difficult to detect, may be responsible for amphibian declines observed in the Lassen region.

We have not attempted to cover here all of the factors that have been implicated or considered in amphibian declines, and that may have impacted the Lassen region, but have mentioned a few. We do know that the Cascades frog is in immediate danger of extirpation from the entire region. The significance of the situation is amplified in light of recent genetic work revealing that gene flow in the Cascades frog is restricted to short distances and that populations in California are genetically distinct from Oregon and Washington populations (Monsen and Blouin 2003 and 2004). While nonnative fish may have been one of the stressors that contributed to the decline, the sequence of events described previously suggests that there must have been some other factor, or group of factors that came together, in the late 1970' or 1980's, to accelerate the decline of the

Cascades frog in the Lassen region, resulting in the near extirpation that had apparently occurred by 1989.

## **Recommendations**

Some land managers in California are evaluating the utility of fish eradication projects. Fish stocking at LVNP was terminated twenty-five years ago, and most (or all) of the localities that are likely to revert naturally to a fishless condition have already done so. Fish eradication of many of the remaining extant localities using gill nets may not be feasible. However, there may be potential to eradicate fish at Dream Lake and Ridge Lakes. If there is support for fish eradication projects, we recommend further evaluating the feasibility of fish eradication by gill netting at those sites.

Cascades frogs in the Lassen region have not begun to recover from extremely low numbers first observed in 1989. Human intervention will likely be necessary to avoid the complete extirpation of this species from the region. Because of the large degree of genetic variation between populations of Cascades frog, the genetic stock required to maintain the frog in the Lassen region may itself be in danger of disappearing. For that reason, we recommend initiating a captive breeding program with animals from the Lassen region.

Meanwhile, experimental studies might be helpful in identifying causes of the Cascades frog decline. One experimental approach would be to translocate eggs and/or larvae to replicated enclosures in the Lassen region. By rearing tadpoles under various experimental treatments, and by conducting histology on animals that may not survive, insight might be gained into what makes Lassen inhospitable to Cascades frog. On the other hand, if the control animals were to survive, then perhaps local conditions for

Cascades frog have improved. That would imply that extremely small population sizes and limitations inherent in the frogs' movement patterns could be preventing them from recolonizing suitable habitat. In that case, captive rearing with reintroductions might be warranted.

Whether populations of long-toed salamander in LVNP are stable is not clear. In light of multiple observations of dead animals, future monitoring of long-toed salamander populations may be warranted. Although the test for chytrid fungus that we performed on one dead animal was negative, ranaviruses (Family: Iridoviridae) are another type of disease recently associated with declining amphibian populations, including other ambystomatid salamanders (Chinchar 2002). Researchers in James Collins' laboratory, at Arizona State University, have successfully isolated ranavirus from tiger salamanders (*Ambystoma tigrinum stebbinsi*), in association with die-offs (Jankovich et al. 1997). This group should be contacted regarding any future salamander die-offs at LVNP, and moribund (preferred) or dead animals should be collected and stored frozen (preferred) or in alcohol for analysis.

Although the distribution and abundance of amphibians observed during this investigation does not represent true "baseline" conditions, since significant changes have clearly occurred prior to our surveys, our results do provide a benchmark by which the status of amphibians in LVNP and the proximal wilderness areas can be measured in the future. Similar surveys conducted in subsequent years should be used to monitor potential changes in species' distribution and abundance. If declines in our protected

areas are occurring as a result of activities initiated outside the boundaries of the protected areas, novel partnerships and unprecedented cooperation may be necessary to fill some of the knowledge gaps that exist, and to begin reversing the declines.



## **Acknowledgements**

Many current and former members of the RSL Herpetology Research Group, including Don Ashton, Miranda Haggarty, Jamie Bettaso, Justin Garwood, Garth Hodgson, Nate Nieto, Kia Ruiz, and Clara Wheeler, contributed at various points in this investigation. Database management by Becky Howard and GIS support by Ron Knickerbocker was, once again, invaluable. We appreciate the full cooperation and logistical support provided by personnel at Lassen Volcanic National Park, including Nancy Nordensten and Jonathan Arnold. Southern Oregon University students John Speece, Kate Meyer, Jesse Goldstein, and Lyndia Hammer were members of the field crew in LVNP. This investigation was supported financially at LVNP by grants from the National Park Service to RSL (NPS Interagency Agreement No. F8485040013) and by cooperative agreement CA9320A004(J9320020026) between the National Park Service and Southern Oregon University. Research at Caribou and Thousand Lakes wilderness areas was financially supported, in part, by Region 1 of CDFG. We would like to thank both CDFG and the Klamath Network for generously loaning us field equipment, and Jess Morgan, of UC Berkeley, for running PCR analysis on our long-toed salamander.



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## Appendix A

### 2004 Lassen High Lakes Inventory Protocol and Blank Data Sheets

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May 13, 2002 (Revised June 2004 by Jonathan E. Stead)

Amended from 2001 Sierra Lakes Inventory Data Sheet Instructions by Roland A. Knapp and 2002 CDFG Fish and Amphibian Inventory Data Sheet Instructions Version 3.0

#### Overview

Fill out a separate data sheet for **every** lake and pond, regardless of how un-lake like the site is. If the site is dry, frozen, is part of another sampled water body, or is a widening of a stream (i.e., there is a current flowing through the site), fill out the top portion of the first page of the datasheet, indicate why a full datasheet was not filled out (e.g. pond was dry) and leave the rest blank. On the map portion of the datasheet, indicate why a full datasheet was not filled out (e.g., "pond was dry"). If you encounter ponds not shown on the 7.5' maps, fill out a data sheet if they contain fish, amphibians, and/or fairy shrimp. Puddles (< 3 or 4 m<sup>2</sup>) without a Site ID should be checked for fairy shrimp and amphibians, but a data sheet need not be filled out. It is critical that all relevant portions of each data sheet be filled out, and that non-relevant portions be indicated as such, not simply left blank. Remember, if the data sheet is improperly filled out, the visit was a complete waste of time and money. Meadows and marshes should always be surveyed. When you visit non-lake habitat such as marshes that contain extensive ponded water, fill out a single data sheet for the entire area.

If you make incidental observations (e.g., fish or amphibian presence) in habitat that does not contain any ponded water (e.g., streams), record the information in a notebook and give this notebook to the data coordinator at the end of the field season. For notebook observations, always record UTM coordinates when describing the location. Record all observations in with a 0.5 mm lead **pencil**.

#### General lake description

**Site ID:** This is a **critical** number, as it will be used to link the data sheet to a particular body of water and to identify all samples. This ID will be color GIS maps available for crews to take into the field. Check the Site ID carefully before recording it on the data sheet. If you encounter a lake or pond that is not shown on the map, or a marsh or meadow that does not have a site ID, its site ID will be the number of the nearest lake or pond that has a site ID plus a double digit decimal place identifier (e.g. 70377.01). Additional Site ID's for nearby unnumbered lake features will be made using consecutive numbers (e.g., 70377.02, 70377.03).

**Date:** Write as day-month year (10-Jun-01) and always use the three-letter abbreviation for month.

**Crew members:** Record the first initial and full last name of all crew members. This is important to ensure that we can contact a particular individual if there are questions about data collected for a particular site.

**Topographic map:** Record the name of the U.S.G.S 7.5' topographic map that contains the lake feature.

**Topo year:** Record the year of the particular map used for field navigation and distance measurements. The year for the topo base of the maps we are using is 1992..

**Lake name:** Obtain lake names from the 7.5' topographic map. If the lake feature is unnamed, put a line through the space. Do not write "unnamed".

**Planning Watershed:** The watershed name for all lakes is given on the "Lakes Checklist". Do not use the name of the outlet creek given on the 7.5' map as the watershed name because this may not be a complete description.

**Wilderness Area:** Lassen V.N.P.

**Elevation:** Record the elevation from the 7.5' map, or from the GPS. On the data sheet, circle the units used (m or ft). Although elevations generally will be shown in feet, some maps give elevations in meters. If the exact site elevation is not given, record the average elevation of the first contour line below the lake and the first contour line above the lake.

**Location:** This description should always be provided for unnamed sites, and must be detailed enough to allow someone not familiar with the area to pinpoint the lake on a topographic map. This information is particularly critical for unnamed lake features, where it is used to identify lakes for which the incorrect Site ID was recorded on the datasheet. At a minimum, give the distance and the compass direction from the site to two nearby prominent named geographical features (e.g., lakes, peaks, etc.). Lake and peak names, distances, and compass directions should be taken from 7.5' maps. Do not use our site ID numbers as reference points, as they do not appear on regular topo maps. Example: Big Bear Lake 740 m and 280 ° from beginning of outlet to summit of Peak 7140 ft. 1050 m and 140 ° from beginning of outlet to summit of Peak 7014 ft.

**UTM Coordinates:** This is a pair of numbers that are basically x and y coordinates. In our area, they are North and East. These numbers need to be obtained for all survey sites. Use a GPS unit, or alternately a UTM grid laid on the 7.5' map, to obtain the UTM coordinates. Take your GPS reading at the outlet, when possible. Record the location of your GPS point on your sketched map of the site. Make sure your GPS is set up to NAD 27. Record the 7-digit UTM North coordinate in the "UTMn" section of the data sheet. Record the 6-digit UTM East coordinate in the "UTMe" section of the data sheet. These coordinates are critical as they will be used to locate the lake feature on the Geographic Information System.

**UTM error:** Record the UTM error (in feet) that is displayed on the GPS unit as the UTM's are being displayed.

**Site type:** Enter the appropriate letter to represent the water type category for the site. If the site is a waterbody that is greater than 0.5 hectares (ha), write "L" for lake. If the site is a waterbody that is less than 0.5 ha (5,000 square m) and has a maximum depth greater than 1.0 m, write "P" for

permanent pond. If the site is a waterbody that is less than 0.5 ha and has a maximum depth less than 1.0 m, write “T” for temporary pond. If the site is a wet meadow, even if it contains scattered small ponds and/or a stream flowing through the middle or adjacent to it, write “W” for wet meadow. If the site is a wetland with emergent herbaceous or woody vegetation (not including water lilies) that covers more than 30 percent of the surface for most of the growing season, write “M” for marsh/bog. If the site is a stillwater pool formed by the outflow from a spring at the bottom of the pool, or a seep upslope, write “S” for spring/seep. If the site is an intermittent stream that has dried up into a string of isolated stillwater pools, write “I” for intermittent stream.

**Drainage:** Circle the appropriate category in the drainage section of the data sheet. If the site has at least one inlet and one outlet with flowing surface water that you estimate will flow all summer long, circle “Permanent.” If the site only has dry inlet and outlet channels, or the inlets and outlets have low surface water flow that you estimate will go completely subsurface before the end of the summer, circle “Occasional.” If the site has no evidence of inlet or outlet channels, circle “None.”

**Wind:** Enter the appropriate letter to represent current wind conditions at the site in the wind section of the data sheet. If there is no breeze present, write “C” for calm. If there is an intermittent or steady light breeze present, write “L” for light. If there is an intermittent or steady moderate wind present, write “M” for moderate. If there is an intermittent or steady heavy wind present, as evidenced by white-capped waves on the surface of a waterbody, write “S” for strong.

**Weather:** Enter the appropriate letter to represent the current weather condition at the site. If the sky has less than 5 percent cloud cover, write “C” for clear. If the sky has 5-50 percent cloud cover, write “P” for partly cloudy. If the sky has 51-95 percent cloud cover, write “M” for mostly cloudy. If the sky has 100 percent cloud cover, write “O” for overcast. If it is raining, write “R” and if it is snowing, write “S.”

**Air temperature:** Measure air temperature from the lake shore at 1 m above the lake surface. Record air temperature in Celsius (°C). Temperature should be measured during midday (1100-1500) when possible.

**Air temperature time:** Record the time the air temperature sample was taken in 24-hour time (Example: 1600).

**Water temperature:** Measure water temperature approximately 0.5 m out from shore and 10 cm under the water surface. Record water temperature in Celsius (°C). Temperature should be measured during midday (1100-1500) when possible.

**Water temperature time:** Record the time the water temperature sample was taken using 24-hour time (Example: 1600).

## **Lake characteristics**

The habitat characterization is perhaps the most subjective of the measurements made using this protocol, and we hope to reduce the potentially high observer bias in these larger lakes by having the information collected by a smaller pool of people. Although priority should be given to the crew leader in surveying large lakes, other crew members should survey large lakes if doing so would save time (e.g., if the crew leader is busy surveying another large lake, and there are no small ponds to survey).

**Fish present?:** Circle the appropriate category. If fish are detected in a site, circle “Yes.” If you are certain that fish are absent from a site, circle “No.” If you are unsure whether fish are present in a site due to low visibility from turbid or choppy water, circle “Unknown.”

**Fish species:** Circle the appropriate category. If rainbow trout are present, circle “RT.” If rainbow trout are present, circle “RT.” If brook trout are present, circle “BK.” If brown trout are present, circle “BN.” If golden trout are present, circle “GT.” If fish are detected but the species cannot be confirmed, circle “Unknown.” If the fish species present is not listed, write other and provide species ID in the “Notes:” field.

**Photos taken:** Circle yes or no depending on whether or not digital photos were taken. Record photo file number displayed on camera. See Appendix for camera setup and additional file naming information.

**Habitat data recorders:** Record the first initial and last name of all people involved in the survey of lake shoreline characteristics.

**Maximum depth:** Measure maximum lake depth with the depth sounder. Do not spend inordinate amounts of time sounding every part of the lake to find exactly the deepest part. By sounding the deepest-looking portion of the lake, you will quickly get a feel for where the deepest spot actually is. Precise measurements of “maximum depth” are not very important in large deep lakes. For some of the large, named lakes in the Park you may use the maximum depth from the table provided, if it would take too long to determine this in the field. However, do not completely neglect to sound the bottom if you are going to be on the lake in a float tube. In shallow lakes (< 5 m deep) a precise depth ( $\pm 0.5$  m) is very important. Plan to take maximum depths when setting or retrieving gill nets. Record the measurement in the right corner of the “Maximum Depth” section of the data sheet. In shallow ponds, if you are not able to electronically sound the maximum depth, obtain it as best you can using a tape measurer. Also circle the category of maximum depth as <1 m, 1-2 m, or >2 m.

**Pace count:** Record the number of 1 m paces that exist between each equally-spaced transect. Estimate a pace count that will obtain 50 transects, as this is a sufficient number to provide an accurate description of the littoral zone of lakes and ponds. For lakes with site IDs, estimated pace counts are provided on the lakes checklist. It is better to obtain more than 50 transects as opposed to less than 50 transects, so be conservative when estimating the pace count. If a site has less than 50 m of shoreline, do not do transects. Simply estimate the percentage of each habitat type for the entire site and record percentages on the data sheet.

**Littoral zone (substrate composition):** While walking around the lake perimeter during the amphibian survey (see [Amphibian/reptile surveying](#), below), stop after a set number of paces (pace count) and categorize the substrate at the lake edge. Categorize the substrate along a 1 m wide imaginary transect line starting at the lake edge, extending perpendicular from shore, and lying along the first 3 m of the lake bottom. (When at a transect where there is less than 3 m of water from the shore to the center of the lake, categorize the transect using whatever length is present from shore to the center of the lake. When near the arm of a lake where there is less than 3 m of water from the shore to the opposite shore, use half of that distance to categorize that transect.) Put a dot in front of the substrate category that occupies the greatest proportion of the imaginary transect line. The dimension associated with each substrate category is the particle diameter (e.g. silt particles are < 0.06 mm in diameter; sand particles range from 0.06-2 mm in diameter; gravels range from 2-32 mm in

diameter; pebbles range from 32-64 mm in diameter; cobbles range from 64-256 mm in diameter; boulders are > than 256 mm in diameter; and bedrock is a large solid piece of rock embedded into the shoreline. Use the dot-line method of recording the number of "hits" in each substrate, instead of the more typical four vertical lines and a slash. The dot-method is much more space-efficient and easy to read. Record the total for each transect category in the box at the lower right of its field.

If a lake or pond is so small that it would be difficult to obtain 50 transects, e.g., less than 50 m of shoreline, estimate and record the percentage of littoral zone dominated by each substrate category, and enter "NA" in the "total transects" section for each category. Also estimate and record the percentage of shoreline where wood/shrubs and aquatic vegetation are present, and enter "NA" in the "total transects" section for wood/shrubs and aquatic vegetation. Always walk around the lake first to conduct the amphibian survey and walk around a second time to measure substrate composition.

**Shoreline terrestrial substrate composition:** At each of the littoral zone transects, also record the dominant substrate along an imaginary line starting at the lake shore and running for 1.5 m perpendicular and away from the lake shoreline. This transect should begin at the lake shore, and go 1.5m away from the water body. Even if the pond has dried way down, begin the transect at the lake shore (normal high water). We are interested in the habitat conditions surrounding the lake, and are not interested in the silt likely present between a drying pool of water and its normal shoreline. Record the total for each transect category in the box at the lower right of its field. As with littoral zone substrate composition for very small sites, it is permissible to estimate the terrestrial substrate composition by size category visually, and then to record your estimates as percent values for each size category (make sure the total of all substrate categories equals 100%).

**Depth at 1.0 m:** At each transect estimate the water depth at 1.0 m in from the shoreline and record in the appropriate depth category box. Record the total for each transect category in the box in the lower right of its field. As with littoral zone substrate composition for very small sites, it is permissible to estimate the littoral zone depth by depth category visually, and then to record your estimates as percent values for each depth category (make sure the total of all depth categories equals 100%).

**Transects with aquatic vegetation:** In addition to categorizing the substrate type at each transect, record the presence or absence of aquatic vegetation at each spot (record hits using the dot-line method). Record aquatic vegetation as present if you detect live herbaceous vegetation growing out of the littoral zone substrate. Use the 10% rule – if vegetation occupies 10% of the transect, then record the presence of vegetation. If less than 10% of the transect has vegetation, consider vegetation absent. This is necessary because if you look closely enough vegetation may always be present, but a tiny filament of grass is not what we mean here. Record the total for each transect category in the box at the lower right of its field.

**Transects with wood or shrubs:** In addition to categorizing the substrate type at each transect, also record the presence or absence of dead or live wood at each transect (record hits using the dot-line method). Record wood as present if you detect downed wood on top of the littoral zone substrate, live shrubs growing out of the littoral zone substrate, or shrub branches drooping in to the littoral zone water column. Again, use the 10% rule. Record the total for each transect category in the box at the lower right of its field.

**Total transects:** Record the total sum of all littoral zone substrate transects. Do not include the terrestrial substrate transects, the transects with wood/shrubs, or the transects with aquatic vegetation in this total, as these are additional measurements at existing transects.

**Evidence of spawning:** This field is for the presence or absence of spawning in the littoral zone of a lake or pond, not including the inlets or outlets. Circle the appropriate category. If spawning fish are detected in the littoral zone, circle “Spawning Fish.” If redds are detected in the littoral zone, circle “Redds.” If fry are detected in the littoral zone, circle “Fry.” If no evidence of spawning is detected in the littoral zone, circle “None.”

**Site length:** Visually estimate the average length of the site.

**Site width:** Visually estimate the average width of the site.

**Shoreline searched:** Record the length of shoreline searched for animals (in meters) during the amphibian/reptile survey. In many cases you will be able to search the entire shoreline, and thus the shoreline searched would equal the entire shoreline length. However, if you had to leave the shoreline to go around cliffs or other obstacles, subtract these distances from the total shoreline length to estimate the shoreline searched. Shoreline length can be determined by multiplying your pace count by the number of transects conducted.

**Shallow water habitat:** While conducting the littoral zone shoreline survey, keep track of the distances (in meters) in a perpendicular line from the shoreline out to a depth of 1.5 m. At the end of the survey, estimate the average distance from the shoreline to 1.5 m in depth, and multiply this distance times the shoreline length to estimate the amount of amphibian habitat in square meters. Record the amphibian habitat in square meters.

## **Amphibian/reptile surveying**

We will be conducting amphibian and reptile surveys at all bodies of waters shown on 7.5' topographic maps and at sites not shown on the map but found during surveys and while traveling between sites.

**Amphibian observers:** Record the first initial and last name of all people involved in the amphibian/reptile survey. This survey typically is conducted by a single observer, and this observer generally will be the same person who conducted the survey of lake shoreline characteristics.

**Survey start time and end time:** Record the time at which the amphibian/reptile survey began and ended using 24 hr time. The start time is the time the amphibian/reptile survey began, not the time you arrived at the site.

**Total survey duration:** Record the total time spent searching for amphibians/reptiles. Do not include time spent surmounting lake-side obstacles (e.g., cliffs), identifying specimens, or recording notes. If two people survey the same site by walking in opposite directions around the lake perimeter, the total survey duration should include the time spent surveying by each person. Record time in minutes, and round off to the nearest whole minute (ex: 42).

**Amphibian/reptile detections:** To conduct an amphibian/reptile survey, walk **slowly** around the perimeter of the site, visually scouting for amphibians and reptiles near the shoreline, and counting the number of adults, sub-adults, larvae, and egg masses you find of each species. Use the sterilized D-net or aquarium net to catch amphibians for identification if necessary. Record detections in the bottom

section of page 1 of the data sheet. Each species/life stage/survey method combination detected should be recorded in a separate row (e.g., HYRE adults detected **visually** during the timed survey in one row, and HYRE adults detected **aurally** during the timed survey in another row). In addition, each species/life stage/survey method combination detected incidentally (e.g., detected before or after the timed survey) also should be recorded in a separate row.

**Species:** Species abbreviations are given abbreviation cheat sheet.

**Life stage:** Life stage abbreviations are “A” for adult, “S” for sub-adult, “M” for metamorph, “L” for larvae, and “E” for egg masses.

**Tally:** Record detections using the dot-line method in the tally section.

**Total:** Record the total for each row in the total section.

**Survey method:** Circle the method used. Most detections will be “visual” and some will be “aural” (e.g., Pacific tree frog adults heard calling but not visually detected would be recorded as an aural detection). In addition, circle “Dip Net” if you use a dip net to catch an individual of a species/life stage combination for identification. Consult the field guide provided for adult, larval, and egg mass identification. Further, circle “Tissue” if you collected tissue samples from individuals in that species/life stage combination

**Incidental?:** Circle the appropriate category. For each species/life stage/survey method combination detected during the timed survey, circle “No.” For each species/life stage/survey method combination detected incidentally (e.g., before or after the timed survey), circle “Yes.”

**Comments:** Record any interesting observations made during the survey (e.g., Cascades frog larvae found only in shallow lagoon on NW side of lake). Also record locations of interesting observations on the map of the lake that you draw (see below).

**Tissue samples:** For sites that contain  $\geq 100$  Cascade frog larvae and/or adults and that are separated by  $\geq 1$  km from other such populations in the same drainage, we will be collecting several tissue samples for a study of the genetic structure of this species in California. We also will collect tissue samples from sites that contain  $\geq 100$  long-toed salamander larvae and/or adults and that are separated by  $\geq 1$  km from other such populations in the same drainage. To collect tissue samples from a site, use the dip net to capture 5 larvae. Sterilize the small scissors by immersing the blades in a cryo-vial filled with 95% ethanol. While gently restraining each larva individually in the net bag with your fingers, clip a 0.5 cm section from the tip of the tail using the small scissors, and release the larvae alive. Place these tissue samples into the same cryo-vial that the scissors were sterilized in, with an internal label containing the date, the Site ID, and the drainage name (in pencil). Screw the top of the vial on tightly. Record the number of tissue samples collected in the “Comments” section for that species/life stage combination. To simplify the process of determining whether a population is  $\geq 1$  km away from the last population (of the same species) from which a collection was made, on the topographic map write “(RC)” next to the lake ID on the map from which frog collections were made, and “(AM)” next to the lake ID on the map from which salamander collections were made. If you are surveying a drainage in which Cascade frog or long-toed salamander populations are rare and/or small, collect a tail tip tissue sample from one larva from each of the larger populations that are separated by  $\geq 1$  km from similar populations and release the larva alive. Label the cryo-vial as described above.

**Chytrid samples:** Recent studies indicate that a chytrid fungus is the likely proximate cause of amphibian declines in several parts of the world. Little is known about this fungus, although we know that it is occasionally found on mountain yellow-legged frogs in the Sierra Nevada. When the fungus attacks the larvae, it deforms their mouthparts. Therefore, for all sites that have Cascades frog larvae, when amphibian surveys are completed or a break can be made without confusing the survey, capture approximately 10 larvae with the dip net and inspect their mouthparts for deformities using a 10x hand lens. If you collected tissue samples, the five larvae used for this should also be used for the mouthpart inspection. Record the number of larvae sampled and the number of these with malformed mouthparts. If you find any larvae with deformed mouthparts, voucher one with the most severe deformities. To voucher the tadpole, euthanize it by emersion in a solution of 10% ethanol. Then take a tissue sample by clipping off the last 0.5 cm of the tail and preserving it in a solution of 95% ethanol. Euthanized animal will then be preserved in a solution of 10% formalin (or 95% ethanol if formalin is not available) in a small nalgene bottle. Fill out a chytrid sample form in pencil and make sure to record the names of collectors, species and life stage of the animal, date and time, location, type of habitat, method of preservation, and any abnormal behavior or appearance you may have noted in the animal prior to being vouchered. Place the label in with the vouchered specimen then put the container in a ziplock and clearly label the bag with a sharpie as a chytrid specimen bag. Release the rest of the larvae back into the lake after you have completed your inspections.

**Fairy shrimp:** During the amphibian survey, be on the look out for schools of fairy shrimp. The distribution of these 2-3 cm crustaceans is poorly known, so we are interested in describing localities. Look for them in all bodies of water you sample. When walking around a lake, take a few minutes to also look in small pools and ponds adjacent to the lake. If you find fairy shrimp during the survey record them as you would amphibian detections. Use “STSE” for *Streptocephalus sealii*, and “FASH” for unidentified populations. You do not need to try to count the fairy shrimp, but make a guess on the order of magnitude (i.e. 10, 100, 1000, 10,000 etc). Be specific in your location descriptions! On the lake map you've drawn, indicate the locations of fairy shrimp populations, and provide a brief description of these locations (e.g., "1 m<sup>2</sup> pool 0.5 m from lakeshore on N side of lake 70675, pool is 10 cm deep"). When mature fairy shrimp are encountered (1.5-3 cm long, females carrying eggs) collect approximately 10 adults (collections at a given site should not exceed 10 individuals, or 10% of the population, whichever is smaller). Try to collect a mix of males and females. Males are used to key the animals to species, but there is some important work to be done with egg bearing females too. Preserve the fairy shrimp in a vial using 95% ethanol. Make an internal label out of a page from your notebook. The label should contain the date, your name, the Site ID, and the drainage name (in pencil). If there are numerous populations of *Streptocephalus sealii* (the most common fairy shrimp in the region) in the same watershed, and you can confirm that they are in fact the same species, collections from all populations are not necessary. If you do not know what species you have (and the shrimp appear to be mature), always make a collection.

**Drawing of lake perimeter:** Based on the 7.5' map, draw the lake perimeter. Add the numbered inlets and outlets from the data sheet. Inlets should be indicated with arrows pointing toward the lake, and outlets should be indicated with arrows pointing away from the lake. If you find in-lake spawning areas or other areas of interest (concentrations of amphibians, locations of adjacent ponds containing fairy shrimp, etc.), indicate these on the map. Also indicate general terrestrial habitat types found around the lake (meadows, talus fields, etc.). Include the deepest point of the lake on the map, and indicate if one portion of the lake is much shallower, or deeper than the rest indicate such. Include the location of the gill net and GPS point on the map.

## Meadows

We do not have a separate protocol for surveying meadows. Instead, we apply our regular protocol to meadows, as best we can. Fill out the header information as you normally would. Do a timed amphibian survey, focusing on shorelines and aquatic and wadeable habitat. Do not completely ignore the rest of the meadow, however. Run the stopwatch throughout. For the habitat survey, we do not do transects in meadows. Estimate the percentage of habitat types for the entire meadow and provide percentages, similar to what you would do on a pond with less than 50 m of shoreline.

Additionally, there is a box at the bottom right hand corner to be filled out when surveying meadows. These parameters should be visually estimated or paced, if necessary. When added together, the dry length and wet length should equal the total Site Length, as recorded above the “Notes:” field of the data sheet. Similarly, the dry width and wet width should cumulatively equal the Site Width.

**Dry Length:** Record the length of the portion of the meadow that is dry.

**Dry Width:** Record the width of the portion of the meadow that is dry.

**Wet Length:** Record the length of the portion of the meadow that is wetted.

**Wet Width:** Record the width of the portion of the meadow that is wetted.

**Lentic Area:** Estimate the area of all standing water in the meadow.

**Lotic Area:** Estimate the area of all flowing water in the meadow.

## Inlet/Outlet surveys

**IOSurveyNo:** Leave blank – for office use only.

**Inlet/Outlet:** Circle “inlet” for each inlet survey, or circle “outlet” for each outlet survey.

**Inlet/Outlet #:** Number each inlet and outlet beginning with number 1 (e.g., a site with 2 inlets and one outlet will need to have 3 inlet/outlet sections filled out; the inlets would be # 1 and # 2, and the outlet would be # 3).

**Width and depth of inlets:** While walking the lake perimeter, estimate and record the bank-full width and depth of each inlet in centimeters even if inlet is dry. Inlets generally are widest at the point at which they enter the lake, so obtain a width and depth at about 5 m upstream from the lakeshore.

**Width and depth of outlets:** While walking the lake perimeter, estimate and record the bank-full width and depth of each outlet in centimeters. Outlets generally are widest at the point at which they leave the lake, so obtain a width and depth at about 5 m downstream from the lakeshore.

**Area of suitable spawning habitat in inlets and outlets:** For the first 50 m of each inlet and outlet, make a visual estimate of the amount of the streambed between the lake and the first barrier that is suitable trout spawning habitat. The amount of spawning habitat should be recorded in terms of the number of square meters of stream bottom with the following characteristics: gravel 0.5-4 cm in diameter and not cemented into the streambed, water depths of 10-50 cm, and water velocities of 20-60 cm/s for successful spawning.

**Evidence of spawning in inlets and outlets:** Check the first 50 m of each inlet and outlet for evidence of spawning. This could be spawning trout, redds (nests), or newly-hatched fry (20-30 mm). Redds are often very obvious, being patches of freshly cleaned gravel 0.5-1 m in length. If you aren't sure if what you are seeing is in fact a redd, dig into the downstream portion of the disturbed gravel while holding a net downstream. If it is a redd, you should find eggs in the net after disturbing the gravel. For each inlet and outlet, circle all types of evidence that you find. If you don't find any evidence of spawning, circle "None".

**Area of in-lake spawning habitat at inlets and outlets:** Estimate the amount of suitable spawning habitat (using the spawning habitat criteria given above) at the mouth of each inlet and outlet. Look for the presence of spawning trout and completed redds.

**Fish present? in inlets and outlets:** Record whether there are fish present in each inlet and outlet stream by circling "Yes" or "No" for each stream. If the stream structure is such that seeing fish would be difficult and you don't see any fish, circle "Unknown" for that particular stream.

**Barrier? in inlets and outlets:** A barrier is an obstacle that is impassable to fish movement. Barriers are falls > 0.75 m high if there is no pool at the base, falls > 1.5 m if there is a pool at the base, or steep cascades higher than approximately 1.5 m. Logjams can float during high water, and generally should not be considered barriers. Because fish can often get over remarkable obstacles, be conservative in what you call a barrier. Record whether there is a barrier present in each inlet and outlet stream by circling "Yes" or "No" for each stream.

**Distance to first barriers on inlets:** Pace off 50 m of each inlet, recording the distance to the first impassable barrier that a fish swimming upstream from the lake would encounter. The barrier location should be recorded as the # of meters from the lake. If there are no barriers on the first 50 m of an inlet, write "none" (**do not write 0**).

**Distance to first barriers on outlets:** Pace off 50 m of each outlet, recording the distance to the first barrier that a fish swimming upstream toward the lake would encounter. The barrier location should be recorded as the # of meters from the lake. If there are no barriers on the first 50 m of an outlet, write "none" (**do not write 0**).

**Barrier type:** Circle the appropriate descriptor. Circle "Waterfall" for a vertical streambed barrier; circle "Cascade" for a very steep (but not vertical) streambed barrier; circle "Subsurface Flow" for a barrier where surface water goes beneath the ground; circle "Snow" for a snow bank barrier. "Circle "Other" for all other barriers.

**Barrier height and length:** If a barrier is present, record barrier height in meters as the vertical height of the barrier, and barrier length in meters as the horizontal length of the barrier. For example, a 2.0 m high cascade that covers a horizontal distance of 5.0 m would be written as 2.0 m / 5.0 m.

**Flow rate:** Circle the appropriate descriptor. Circle “Low” for slow moving surface water flow; circle “Moderate” for moderate flow rates; circle “High” for fast moving surface water; circle “None” for streams with stagnant or no surface water flow.

**Gradient:** Estimate the gradient of the slope that each inlet and outlet flows through, and record as a percent (X m / 100 m) in the gradient % section of the data sheet. While you are conducting your stream surveys, keep track of the number of meters that the slope rises (for inlets) or drops (for outlets) over the course of your 50 m survey. For example, if an inlet rises 5 m in height over 50 m in length, the percentage gradient would be 10 %.

**Substrate (dominant):** Estimate the three dominant substrate categories over the first 50 m of each inlet and outlet. Record the most dominant substrate in the “Substrate (1)” section, the second most dominant substrate in the “Substrate (2)” section, and the third most dominant substrate in the “Substrate (3)” section. Category abbreviations are located beneath the substrate section of each inlet/outlet form. Categories include silt, sand, grav (gravel), pebb (pebble), cobb (cobble), boul (boulder), detr (detritus), wood, and vege (herbaceous vegetation).

**Amphibian/reptile detections:** During each inlet and outlet survey, visually scout for amphibians and reptiles and count the number of adults, sub-adults, larvae, and egg masses you find of each species. Record detections in the species section for that inlet or outlet. Each species/life stage combination detected during the timed survey should be recorded in a separate row (e.g., HYRE adults detected in one row, and HYRE sub-adults detected in another row). In addition, each species/life stage combination detected incidentally (e.g., detected before or after the timed survey) also should be recorded in a separate row.

**Length searched:** For each inlet and outlet surveyed, record the total length from shore (in meters) that was searched for animals and habitat.

**Survey start time and end time:** For each inlet and outlet surveyed, record the time at which the survey began and ended using 24 hr time.

**Total survey time:** Record the total time spent searching for amphibians/reptiles. Do not include time spent surmounting stream-side obstacles (e.g., cliffs), identifying specimens, or recording notes. Record time in minutes, and round off to the nearest whole minute (ex: 42).

## **Fish surveying**

We will be conducting fish surveys at all bodies of water shown on 7.5' topographic maps and at sites not shown on the map but found during surveys and while traveling between sites.

**Fish survey:** Record whether fish were surveyed visually or using gill nets. Except for small, shallow (<2 m) bodies of water in which the surveyor can see the entire lake bottom, we typically sample fish populations using gill nets. If there is any question as to whether fish are present in a lake, set a net. The decision whether to set a gill net in a shallow pond is up to the crew leader, but keep in mind that fish can live in some very marginal habitats. If only a visual fish survey is needed (e.g., because the lake is < 2 m deep and you can see the entire bottom), you need not fill out the fish capture datasheet.

**Justification:** If you surveyed for fishes visually, provide a brief justification as to why you chose this method (e.g., "pond only 50 cm deep, entire bottom visible, no fish seen").

**Site ID:** If you are setting a gill net to survey a fish population, fill out the fish capture datasheet. First, record the Site ID again. This identifier will ensure that both sheets of the datasheet are associated with the correct lake. Make sure that the Site ID you record is the correct one and matches the Site ID on the first page of the datasheet.

**Description of net location/setting nets:** Circle the appropriate location and provide a brief description of the area in which the net was set ("Comments"). Our fish survey methods are designed to provide an accurate representation of fish species composition and size structure in lakes and ponds, as well as provide an estimate of catch per unit effort (CPUE) at each location. In order to quantify the size structure of each fish species present at a particular location, we need a sample of at least 20 fish, and preferably not more than 50. Obviously, in lakes that have a very small fish population, capturing even 10 fish may not be possible. Nets should be stored and transported in stuff sacks to keep them from getting tangled and to keep them out of the sun. In order to get reasonably accurate measures of CPUE, all net sets must be made for a similar amount of time. We will set one net in each lake for a minimum of 4 hours and maximum of 10 hours. Nets can be set at any time of day, since we should be able to statistically remove any time effect (e.g., increased catch rates at night). To minimize logistical problems and safety hazards, however, do not pull nets at night. Time your net sets appropriately. For example, don't set a net at 6 PM, since this would mean either pulling the net at 10 PM or waiting until morning and exceeding the 10-hour maximum set duration.

Gill nets should always be set at the lake outlet, if present and if conditions allow. If an outlet does not exist, or is located in an area that is difficult to net (water <2 m deep, log jams, etc.), set nets at the inlet. If an inlet is not present or is not suitable, set the net in a suitable location anywhere along the lake shore. Draw the net set location on the sketched site map. If possible, choose an area that is 3-8 m deep. Before setting a gill net, submerge the entire net (still contained on the handle); dry nets are much more susceptible to tangling. To set the net, put a small rock into each of two mesh bags and clip one bag to the shore end of the net (end with loop). Get in your float tube and wedge the bag between rocks at the lake shore and pull on it gently to ensure that it is firmly anchored. With the net lying across the float tube (lead line on your left and net handle in your right hand or vice versa), paddle backwards slowly while feeding out the net. The net should be set perpendicular to the shore. If you encounter a tangle while feeding out the net, shake the net. Do not pull on the net as this will often tighten the tangle. Shaking will nearly always rid the net of the tangle. When you get to the end of the net, attach a float to the handle and then clip the second bag to the bottom of the net. Paddle backwards until the net is taught, and then drop the bag. Record the time when you finish setting the net.

After 4-10 hours, retrieve the net by pulling the mid-lake end of the net up by the float. Detach the float and the bag. Pull the net toward you, placing the float line on one side of the float tube and the lead line on the other. Continue pulling in the net until you reach the shore. Remove the second bag. To carry the net to an area for fish removal, cradle the net over your arms keeping the lead line on one side and the float line on the other. Lay the net down in a meadow or on a sandy flat (a meadow is preferable, but nearly any place will work; stay away from areas with lots of woody vegetation, pine needles, pine cones, and sharp rocks since they will get snagged in the net). Spread out the first 10 feet of net and remove the fish. After removing all fish from the first 10 feet of net, spread the next 10 feet of net and fold up the first 10 feet. Continue until you have removed all fish from the net. Restrung the net onto the handle, rinse the net in the lake, dry the net in the shade, tie the net in a knot to prevent tangling, and stuff it into a sack.

If no fish were captured, write "no fish" across the fish portion of the data sheet. If fish were captured, record the species, length, weight, and sex of all fish. Species abbreviations are given on the abbreviation cheat sheet. Measure fish using the vinyl tape laid out on the ground. Measure fish total

lengths to the nearest mm. Weigh fish using a Pesola spring scale. Before weighing fish, ensure that all debris (small rocks, etc.) are removed from the fish. Use the 100 g scale for all fish <100 g, and the 1 kg scale for larger fish. If someone on your crew is able, note the general contents of fish stomachs (e.g., chironomid pupae, terrestrial insects, etc.). If you encounter a lake that contains both fish and amphibians, look through the fish stomachs very carefully for amphibian remains.

Be careful about disposing of fish carcasses, as we don't want the carcasses attracting the attention of backpackers or bears. The best disposal method is to pop the fishes swimbladders, put the fish in a stuff sack, paddle out into the lake until you reach a relatively deep area, and dump them. Burial of fish on land should generally be avoided, as animals can smell the fish and will dig them up.

**Net set time and date:** Record the time when you completed the net setting process, not the time when you started setting the net. Record the time as 24 hr time. Record the date on which the net was set.

**Net pull time and date:** Record the time when you began pulling the net and the date on which the net was pulled.

## **Field review of datasheets**

While in the field, the crew leader should review all datasheets for completeness and clarity. Once review of a datasheet is completed, the crew leader should initialize the field review box at the bottom of the first page of the datasheet.

## **Stream surveys**

Whenever you encounter a stream during your inventory work and associated travel, take a few minutes to note whether there are fish present. If fish are present, try to determine what species are represented. A pair of close-focus binoculars are very helpful for accomplishing this. Record your observations in a notebook.

## **Public outreach**

During our surveys, we will undoubtedly be asked many questions by the public. Keep your responses brief and simple. For example, if someone asks what you are doing, inform them that you are conducting an inventory of fish and other aquatic taxa in lakes throughout LVNP. Be aware that potential changes in fish stocking, amphibian declines, etc. are hot button issues.

## **Post trip wrap-up**

When you finish a survey trip and return to the office, crew leaders will put all completed data sheets, attached to the completed site status sheet, in the data sheet in box. Separate your labeled disease, tissue samples, or fairy shrimp by watershed into separate ziplock bags labeled with the words "chytrid", or "tissue" and the watershed from which each sample was collected. Decontaminate your gear if you have not yet done so. Download photos. If any equipment needs to be repaired or replaced, discuss this with the lab coordinator (Jon). Obtain datasheets, sample bottles, and other necessary equipment for your next trip.



Trinity Alps Wilderness Aquatic Survey Data Sheet  
Inlet/Outlet Surveys (to 50 meters) and Drawing

Page \_\_\_\_\_ of \_\_\_\_\_

IOSurveyNo:	Inlet	Outlet	#	Width: (cm)	Depth: (cm)	Fish Present? Yes No Unknown	Barrier? Yes No Unknown	Distance to first barrier: (m)	Flow Rate: Low Moderate High None			
Area of suitable spawning Habitat (m <sup>2</sup> ):			Area of in-lake spawning at Inlet/Outlet (m <sup>2</sup> ):			Start Time:	End Time:	Total Time: (min.)	Evidence of spawning: Spawning Fish Redds Fry None			Gradient %:
Substrate (1):	Substrate (2):	Substrate (3):		Barrier Type: Waterfall Cascade Subsurface Flow Snow Other				Barrier Ht.: (m)	Barrier Length: (m)	Length Searched: (m)		
Silt Sand Grav Pebb Cobb Boul Bedr Detr Wood Vege			Notes:									

Species	Life Stage	Tally	Total	Incidental?	Species	Life Stage	Tally	Total	Incidental?
				Yes No					Yes No
				Yes No					Yes No
				Yes No					Yes No

IOSurveyNo:	Inlet	Outlet	#	Width: (cm)	Depth: (cm)	Fish Present? Yes No Unknown	Barrier? Yes No Unknown	Distance to first barrier: (m)	Flow Rate: Low Moderate High None			
Area of suitable spawning Habitat (m <sup>2</sup> ):			Area of in-lake spawning at Inlet/Outlet (m <sup>2</sup> ):			Start Time:	End Time:	Total Time: (min.)	Evidence of spawning: Spawning Fish Redds Fry None			Gradient %:
Substrate (1):	Substrate (2):	Substrate (3):		Barrier Type: Waterfall Cascade Subsurface Flow Snow Other				Barrier Ht.: (m)	Barrier Length: (m)	Length Searched: (m)		
Silt Sand Grav Pebb Cobb Boul Bedr Detr Wood Vege			Notes:									

Species	Life Stage	Tally	Total	Incidental?	Species	Life Stage	Tally	Total	Incidental?
				Yes No					Yes No
				Yes No					Yes No
				Yes No					Yes No

Drawing of lake perimeter, locations of vegetation, woody debris, inlet, outlet, maximum depth, GPS location, gill net (if set), and in-lake spawning areas:

# Wilderness Aquatic Surveys Collection Form

## Inlet/Outlet Surveys (to 50 meters), Continuation Sheet

Site ID: \_\_\_\_\_ Page \_\_\_\_\_ of \_\_\_\_\_

IOSurveyNo:	Inlet	Outlet	#	Width: (cm)	Depth: (cm)	Fish Present? Yes No Unknown	Barrier? Yes No Unknown	Distance to first barrier: (m)	Flow Rate: Low Moderate High None				
Area of suitable spawning Habitat (m <sup>2</sup> ):			Area of in-lake spawning at Inlet/Outlet (m <sup>2</sup> ):		Start Time:	End Time:	Total Time: (min.)	Evidence of spawning: Spawning Fish Redds Fry None			Gradient %:		
Substrate (1):	Substrate (2):	Substrate (3):	Barrier Type: Waterfall Cascade Subsurface Flow Snow Other					Barrier Ht.: (m)	Barrier Length: (m)	Length Searched: (m)			
Silt Boul	Sand Bedr	Grav Detr	Pebb Wood	Cobb Vege	Notes:								
Species	Life Stage	Tally			Total	Incidental?	Species	Life Stage	Tally			Total	Incidental?
						Yes No							Yes No
						Yes No							Yes No
						Yes No							Yes No
						Yes No							Yes No

IOSurveyNo:	Inlet	Outlet	#	Width: (cm)	Depth: (cm)	Fish Present? Yes No Unknown	Barrier? Yes No Unknown	Distance to first barrier: (m)	Flow Rate: Low Moderate High None				
Area of suitable spawning Habitat (m <sup>2</sup> ):			Area of in-lake spawning at Inlet/Outlet (m <sup>2</sup> ):		Start Time:	End Time:	Total Time: (min.)	Evidence of spawning: Spawning Fish Redds Fry None			Gradient %:		
Substrate (1):	Substrate (2):	Substrate (3):	Barrier Type: Waterfall Cascade Subsurface Flow Snow Other					Barrier Ht.: (m)	Barrier Length: (m)	Length Searched: (m)			
Silt Boul	Sand Bedr	Grav Detr	Pebb Wood	Cobb Vege	Notes:								
Species	Life Stage	Tally			Total	Incidental?	Species	Life Stage	Tally			Total	Incidental?
						Yes No							Yes No
						Yes No							Yes No
						Yes No							Yes No
						Yes No							Yes No

IOSurveyNo:	Inlet	Outlet	#	Width: (cm)	Depth: (cm)	Fish Present? Yes No Unknown	Barrier? Yes No Unknown	Distance to first barrier: (m)	Flow Rate: Low Moderate High None				
Area of suitable spawning Habitat (m <sup>2</sup> ):			Area of in-lake spawning at Inlet/Outlet (m <sup>2</sup> ):		Start Time:	End Time:	Total Time: (min.)	Evidence of spawning: Spawning Fish Redds Fry None			Gradient %:		
Substrate (1):	Substrate (2):	Substrate (3):	Barrier Type: Waterfall Cascade Subsurface Flow Snow Other					Barrier Ht.: (m)	Barrier Length: (m)	Length Searched: (m)			
Silt Boul	Sand Bedr	Grav Detr	Pebb Wood	Cobb Vege	Notes:								
Species	Life Stage	Tally			Total	Incidental?	Species	Life Stage	Tally			Total	Incidental?
						Yes No							Yes No
						Yes No							Yes No
						Yes No							Yes No
						Yes No							Yes No

IOSurveyNo:	Inlet	Outlet	#	Width: (cm)	Depth: (cm)	Fish Present? Yes No Unknown	Barrier? Yes No Unknown	Distance to first barrier: (m)	Flow Rate: Low Moderate High None				
Area of suitable spawning Habitat (m <sup>2</sup> ):			Area of in-lake spawning at Inlet/Outlet (m <sup>2</sup> ):		Start Time:	End Time:	Total Time: (min.)	Evidence of spawning: Spawning Fish Redds Fry None			Gradient %:		
Substrate (1):	Substrate (2):	Substrate (3):	Barrier Type: Waterfall Cascade Subsurface Flow Snow Other					Barrier Ht.: (m)	Barrier Length: (m)	Length Searched: (m)			
Silt Boul	Sand Bedr	Grav Detr	Pebb Wood	Cobb Vege	Notes:								
Species	Life Stage	Tally			Total	Incidental?	Species	Life Stage	Tally			Total	Incidental?
						Yes No							Yes No
						Yes No							Yes No
						Yes No							Yes No
						Yes No							Yes No

# Wilderness Aquatic Surveys Collection Form Net Fish Captures (Side 1)

Site ID: \_\_\_\_\_

Page \_\_\_\_\_ of \_\_\_\_\_

Description of net location:			Net set time (hhmm):		Net pull time (hhmm):			
Near Inlet	Near Outlet	Neither	Date (dd/MMM/yy):		Date (dd/MMM/yy):			
Comments:								
Fish #	Species:	Total Length (mm):	Fork Length (mm):	Weight (g):	Sex:	Egg Stage Early: Late: Ripe?	Otoliths:	Comments:
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